EFFECT OF COATING OF MODIFIED STARCH FROM WASTE POTATO ON RESPIRATION RATE AND MICRO-NUTRIENTS OF *Chaenomeles japonica*

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2019

Effect of Coating of Modified Starch from Waste Potato on Respiration Rate and Micro-Nutrient of *Chaenomeles japonica*

A dissertation submitted to Department of Food Technology, Central Campus of Technology Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech in Food Technology

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December, 2019

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Approval Letter

This *dissertation* entitled *Effect of Coating of Modified Starch from Waste Potato on Respiration Rate and Micro-nutrients of Chaenomeles japonica* presented by Prabesh Bhattarai has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in food technology.

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Acknowledgements

I express my deep sense of gratitude to my respected supervisor lecturer Mr. Yadav K.C, Central Campus of Technology, Dharan for his excellent guidance, valuable suggestions and motivation throughout the work.

I express my deep gratitude to Prof. Dr. Dhan Bahadur Karki, Campus Chief, CCT, and Prof. Basanta Kumar Rai, Chairperson, Food Technology Instruction Committee, CCT for providing requisite facilities and encouragement for the work.

I owe a special thanks to my friend Sajal Bhattarai. Likewise, I owe special thanks to Anish Dulal, Samit Karki, Ashish Rai, in particular. I am also grateful to my beloved seniors Prajwal Dipak Pokharel, Ajit Kumar Shah, Pankaj Dahal, Gajendra Bohora, Saroj Ghimire, and my juniors Manish Dahal, Ramu Tiwari, Pratirodh Khatiwada, Ranjan Shrestha, Muskan Saru Magar, Ghanshyam Aryal, Arjun Poudel, Arjun Gautam, Nabin Bhattarai, Sudarshan Gosai, Poshan B.K and Rabu Thapa Magar.

I would like to specially thank Mr. Sachin Basnet and Prajwal Bhandari for their noteworthy help during my thesis. Thanks also goes to all the laboratory and library staffs of Central Campus of Technology, Dharan for their kind co-operation.

Lastly, I am very much indebted to my parents and family members for their constant encouragement, love, inspiration and moral support throughout the work. Without their support, this work would never have seen the light of day.

Date of submission: December 9, 2019

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Prabesh Bhattarai

Abstract

Potato starch isolated from waste potatoes of different *haat-bazars*, hotels and restaurants of Dharan sub-metropolitan city were modified by two techniques, viz. hydrothermal treatment (moisture adjusted to 28% and heated at 110°C for 3 h) and acid-alcohol treatment (treated with 100 ml of rectified alcohol and 20 ml of conc. HCl) to study different physicochemical properties of the extracted starch and films. The films were used to study rate of respiration and retention of micro nutrients (chlorophyll, vitamin A, retinol and β-carotene) in *Chaenomeles japonica* after coating with the different starch suspensions.

Significant effect in functional properties of starches was found by different modifications ($p<0.05$). Acid alcohol treatment improved the oil absorbing capacity, solubility, and iodine affinity of starch. Hydrothermal treatment improved the water binding (WBC), dispersibility, and wettability of potato starch. Non-coated fruit had the maximum rate of respiration—2216.667 mg CO_2/kg/h in day 3 compared to all coated fruits. The rate of respiration significantly ($p<0.05$) decreased with the starch coat in *Chaenomeles japonica.* The chlorophyll had maximum value (2.47521±0.02859 mg/100 g) in non-treated starch plasticized with 45% glycerol and minimum value (0.5246±0.046143 mg/100 g) HTT treated starch plasticized with 55% sorbitol. The vitamin retention was maximum (0.532193±0.05932 μg/100 g) in HTT treated starch plasticized with 55% glycerol. However, HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol were not different form each other. These treatments were however significantly different from the control sample. The treatment that had similar effects on change of both Vitamin A and retinol are HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol. The highest retention of carotene (0.721014± 0.206253 μg/100 g) was observed in HTT treated starch plasticized with 55% glycerol and non-treated starch treated with 55% sorbitol and minimum retention (0.025522±0.00287 μg/100 g) was found in HTT treated starch plasticized with 35% sorbitol. These maximum and minimum values were significantly different from the control and non-coated samples of day one. Waste starch can be utilized—by coating—to increase shelf-life in fruits and vegetables.

Key words: Potato starch, functional properties, rate of respiration, micro-nutrients

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List of Abbreviations

Abbreviations	Full forms
AAT	Acid Alcohol Treatment
ANOVA	Analysis of variance
D	Dispensability
DS	Degree of Substitution
FDA	Food and Drug Administration
HTT	Hydrothermal Treatment
IAS	Iodine Affinity of Starch
LDPE	Low-Density Polyethylene
LSD	Least Significant Difference
MAS	Modified Atmospheric Storage
OAC	Oil Absorption Capacity
PC	Paste Clarity
RA	Retinoic Acid
RAE	Retional Activity Equivalent
WBC	Water Binding Capacity
WVTR	Water Vapor Transmission Rate

Part I

Introduction

1.1 General introduction

Fruits and vegetables are essential in the human diet due to the health and nutritional benefits associated with their intake. However, they are products with a relatively short postharvest life, since they remain as living tissues up until the time they are used for consumption and are prone to physiological and biochemical changes, which can also have physical or pathological origins leading to important economic losses (Palou *et al.*, 2015). The respiration process continues even in their post-harvest life and results in the shorter shelf life if the effective preservative methods are not applied. The use of polymeric films (plastics) for the packaging of the fresh fruits and vegetables is common in the food industries because of their low cost, easy availability, good barrier properties against the respiratory gasses etc. But these packaging materials have adverse effect on the environment due to their non-biodegradability. Therefore, nowadays food industries are focusing on the ecofriendly biodegradable packaging such as edible films and coatings (Menezes and Athmaselvi, 2016).

Synthetic polymer materials have been widely used in every field of human activity during last decades. These artificial macromolecular substances are usually originating from petroleum and most of the conventional ones are regarded as non-degradable. However, the petroleum resources are limited and the thriving use of non-biodegradable polymers has caused serious environmental problems. In addition, the non-biodegradable polymers are not suitable for temporary use such as sutures. Thus, the polymer materials which are degradable and/or biodegradable have been paid more and more attention since the 1970s (Lu *et al.*, 2009).

Both synthetic polymers and natural polymers that contain hydrolytically or enzymatically labile bonds or groups are degradable. The advantages of synthetic polymers are obvious, including predictable properties, batch-to-batch uniformity and can be tailored easily. In spite of this, they are quite expensive. This reminds us to focus on natural polymers, which are inherently biodegradable and can be promising candidates to meet different requirements (Chiellini and Solaro, 1996). Owing to its complete biodegradability, low cost

and renewability, starch is considered as a promising candidate for developing sustainable materials. Many efforts have been exerted to develop starch-based polymers for conserving the petrochemical resources, reducing environmental impact and searching more applications (Araujo *et al.*, 2004).

Potato is most widely used vegetable in the world. Potato peels and its wastes serve as good source of the starch, cellulose, hemicelluloses and fermentable sugars. In potato processing plants, a significant amount of the potato sludge, peels and damaged tubers are obtained as the by-product. Starch dewatering and recovery are being carried out by using centrifuge systems. As a part of waste management, potato peels and potato sludge can be treated for the starch isolation (Arapoglau *et al.*, 2010).

Recently, environmental policy and rising environmental consideration throughout the world have activated changed endeavor in plastic industry to develop new products and development with our environment friendly. Amylose film (paper) can be used in wrapping in confectionaries and commercial food products because these can be easily degraded by bacteria such as *Bacillus* species, *Streptococcus* species and ruminant bacteria of animals as well as many fungal members. (Aburto *et al.*, 1999; Arvanitoyannis *et al.*, 1996; Tang and Alavi, 2011). The main aim of this study is to observe the effects of modified and non-modified starch film coating on respiration rate and retention of nutritional parameters of fruits.

Though, the production of the starch biopolymer is not done in Nepal yet, there is an increasing interest in utilizing renewable resources as food packaging (Bertuzzi *et al.*, 2007a). The use of biodegradable polymers for packaging offers an alternative and partial solution to the problem of accumulation of solid waste composed of synthetic inert polymers. Usually, the film-forming substances are based on proteins, polysaccharides, lipids and resins or on a combination of these. Polysaccharides such as starches, cellulose derivates and plant gums are being studied as edible films and coatings in food packaging and preservation (Debeaufort *et al.*, 1998). Starch films and coatings have been used for various food and pharmaceutical applications. Edible films are viewed as an alternative for increasing the shelf life of fruits and vegetables, protecting them from the effects of humidity and oxygen and thus delaying their deterioration. Films prepared from starches are isotropic, odorless, tasteless, colorless, non-toxic and biodegradable. Edible films and coatings can be prepared

from native and modified starches. The starch films have low oxygen permeability (Forsell *et al.*, 2002a). Starch coatings are nutritious, safe and economic and have been used in the storage and marketing of foods (Avena-Bustillos and Krochta, 1993; Baldwin *et al.*, 1997). The mechanical properties like tensile strength (TS) and percentage elongation of synthetic polymers such as low-density polyethylene (LDPE) and high-density polyethylene (HDPE) are significantly higher than those of the biopolymer films (Cunningham *et al.*, 2000a). However, the latter do have the potential to replace the conventional packaging in some applications. Although the water vapor transmission rate (WVTR) of biopolymers is higher than good barrier materials such as LDP, they are sufficient for short term (hours-days) protection against moisture. The qualities of renewability, degradability, compost-ability, and edibility make such films and coatings particularly appealing for food and non-food packaging applications. Moreover, wide commercialization of biopolymer films will provide a value-added innovative use for traditional agricultural commodities as sources of film forming materials. Amylose is responsible for the film-forming capacity of starch-based films. High amylose induces strong gel network with water after gelatinization and is responsible for film forming capacity of starch-based films. It would result in strong and flexible films due to amylose crystallization (Bertuzzi *et al.*, 2007a).

1.2 Statement of problems

Packaging is key operation for preservation of the food products. Synthetic polymers are widely used for packaging of the food products.

As of 2015, approximately 6300 MT of plastic waste had been generated, around 9% of which had been recycled, 12% was incinerated, and 79% was accumulated in landfills or the natural environment. If current production and waste management trends continue, roughly 12,000 MT of plastic waste were in landfills or in the natural environment by 2050 (Geyer *et al.*, 2017).

Plastic contains chemicals or additive to give it certain properties. Some of the key chemicals are Bisphenol A (negative impact on reproductive systems), Phthalates (endocrine disruptors), and Brominated Flame Retardants (hormone disrupting effects that impairs development of the reproductive and nervous system). As these packaging materials have adverse effect on the environment, so to make ecofriendly and chemical free packaging material the biodegradable coatings/films are emerging in the market. The biodegradable

film can be prepared from polysaccharides such as starches, cellulose derivatives and plant gums.

Almost 10% of the potato is wasted during processing in food industries. From that wasted potato, starch can be extracted and it can be process into biodegradable coatings and films. So, wasted potatoes can be reused for preparation of biodegradable plastics which helps to make ecofriendly environment and also in chemical free packaging material for food commodities.

1.3 Research objective

1.3.1 General objective

The general objective of the study is to study the effect of modified starch coating obtained from waste potato on respiration rate and micro nutrient of *Chaenomeles japonica*

1.3.2 Specific objectives

1. To extract starch from waste potato and its peels.
2. To modify the extracted starch by hydrothermal treatment and acid alcohol modification.
3. To study the functional properties of raw and modified starch.
4. To prepare and evaluate the yield of biodegradable polymer from the extracted starch.
5. To coat starch suspension on *Chaenomeles japonica*.
6. To study the effect of coating on rate of respiration and micro-nutrient (chlorophyll, Vitamin A, Retinol, β-Carotene) of *Chaenomeles japonica.*

1.4 Significance of study

Plastics packaging are non-biodegradable and they cause ecological imbalance and aesthetic deterioration of nature. Incineration may generate toxic air pollution, and satisfactory landfill sites are limited. Also, the petroleum resources are finite and are becoming limited. The environmental impact of persistent plastic wastes is evoking more global concern as alternative disposal methods are limited. Biodegradable packaging materials neither promote any waste disposal problems nor affect the trade and safety of the food product (Sharma, 2000). There is, therefore, a great need to develop environment friendly biodegradable

packaging materials which do not cause environmental pollution. Starch is a natural polymer which possesses many unique properties which can be obtained easily in large quantity from vegetable waste. Some synthetic polymers are biodegradable and can be tailor-made easily. Potato and its products are extensively used in food processing industries, restaurant, hotels and home kitchens. As a part of waste management, starch can be extracted from such wastes and bi-products. Therefore, by combining the individual advantages of starch and synthetic polymers, starch-based completely biodegradable polymers (SCBP) are potential for applications in biomedical and environmental fields. Therefore, it eagerly requires a great attention and needs an extensive investigation of starch biopolymer mainly from vegetable wastes.

Polysaccharides such as starches, cellulose derivatives and plant gums are being studied as edible films and coatings in food packaging and preservation. Starch films and coatings have been used for various food and pharmaceutical applications. Edible films are viewed as an alternative for increasing the shelf life of fruits and vegetables, protecting them from the effects of humidity and oxygen and thus delaying their deterioration. The bi-product of potato can be used for preparation of environmentally friendly bio-films.

1.5 Limitations of study

The research is based on the extraction of starch from waste potato and its peels and preparation of bio-films and coating on fruits. So, the major limitation of the research is as follows:

1. Although starch modification with various methods could be performed, this study cannot estimate the degree of modification, which requires a mass spectrometric analysis.
2. The biodegradation of the starch film cannot be studied due to lack of time.
3. Various properties of biopolymer cannot be studied due to lack of time.

.

Part II

Literature review

2.1 Biodegradable polymers

The level of research devoted to the development of new biodegradable materials, essentially due to the desire to protect the environment has emerged. The growing demand for biodegradable polymers by emerging technologies including tissue engineering and regenerative medicine, gene therapy, novel drug delivery systems, implantable devices and nanotechnology has resulted in the development of a range of biodegradable polymers, mainly based on already known chemistry (Heller and Abraham, 2003). Biodegradable polymer films are not meant to totally replace synthetic packaging films. However, biodegradable and edible films can be satisfactorily used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials (Nelson and Fennema, 1991). Polymers are widely used in the preparation of biodegradable films. These polymers include starches, proteins, PLA (polylactic acid), PHA (poly-hydroxy-alkanoate), cellulose esters, and poly-anhydrides. Starch films and coatings have been used for various food and pharmaceutical applications. Films prepared from starches are isotropic, odorless, tasteless, colorless, non-toxic and biodegradable. Edible films and coatings can be prepared from native and modified starches. The starch films have low oxygen permeability (Forsell *et al.,* 2002a). Starch coatings are nutritious, safe and economic and have been used in the storage and marketing of foods (McHugh *et al.,* 1993). Many efforts have been exerted to develop starch-based polymers for conserving the petrochemical resources, reducing environmental impact and searching more applications. Owing to its complete biodegradability, low cost and renewability, starch is considered as a promising candidate for developing sustainable materials (Lu *et al.,* 2009).

Various bacterial species are found to be amylase producer and they are used commercially also and kinetics of starch film utilization has been studied. (Arvanitoyannis *et al.*, 1996). Bacteria like *Bacillus subtilis, B amyloiquifaciens*, and lactic acid bacteria and fungi like

Aspergillus niger, *Rhizopus niveus* are studied in various researches for the different types of amylase production and starch and starch biofilm biodegradation. Their purified extracted amylase has been studied for the study of digestion activity of different bacteria and fungi (Smith and Lineback, 1976).

2.2 Potato

Potato (*L. Solanum tuberosum*) belongs to Solanaceae family and is the fourth most cultivated crops after wheat, rice and maize the world. It is the good source of: carbohydrates, protein, vitamins, minerals and trace elements. In a Pakistan, there are good climatic environment for the growth of this crops, and Pakistan is self-sufficient in potato production. Potato is being used in different industries as a whole or a part of processing having different products like French fries, potato chips, potato starch, potato powder and potato proteins etc. During the processing operations a reasonable amount of potato solids go into the waste especially the peels of potato, which may be a good source of many bioactive compounds. Potato peel contains fibber, dietary fiber and other carbohydrates that can be further hydrolyzed to produce inulin, oligofructose, lactulose and resistant starch etc. Potato peel has been proved medicinally important as it contains phenolic, polyphenolic compound, anthocyanins, non-anthocyanin flavonoids and glycoalkaloids which are health beneficial having antioxidential and anti-bacterial properties. Many products of industrial importance like amylases, citric acid and prebiotics etc., are being produced by using potato waste as microbial substrate. Moreover, Pakistan is facing energy crises these days and potato waste can be good substrate for biofuel / biogas production. Innovation in technologies for the efficient potato waste management and extraction/ production of value added products from this waste (Ali *et al.*, 2015).

2.3 Starch

Chemically, starch is polysaccharide composed of the elements carbon, hydrogen and oxygen in the ratio of 6:10:5, leading to the molecular formula of $(C_6H_{10}O_5)_n$. A typical starch granule consists of small amounts of proteins, lipids, phosphorus and inorganic materials (Swinkels, 1985b). In addition to these materials, 97-99% of the starch molecule comprises of the polymers amylose and amylopectin. Amylose is a linearly chained molecule, which is connected by α- (1, 4) linkages, and may contain between 500 and 5000glucose units (Galliard and Bowler, 1987;

Wolfrom and Khadem, 1996). Generally, the molecular weight of amylose ranges from 105 to 106. The linear structure of amylose is exhibited in Fig. 2.1

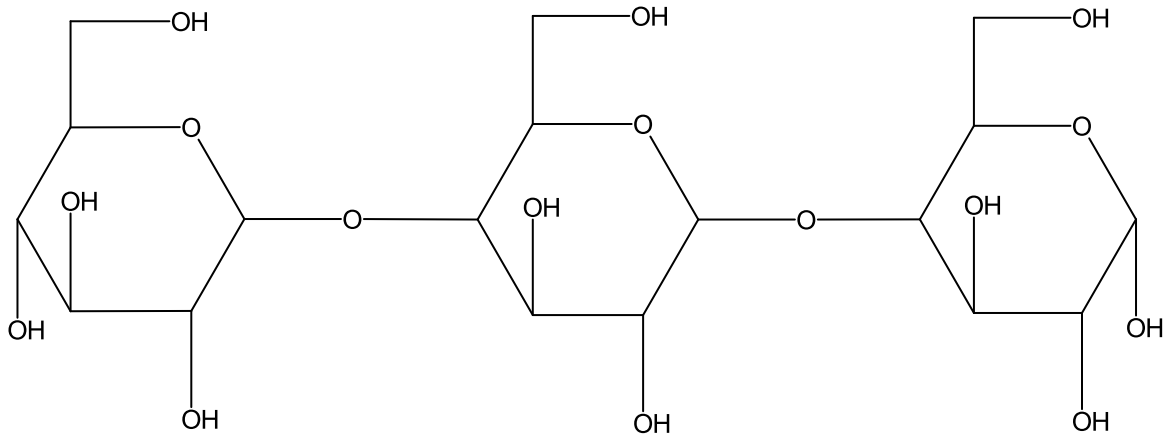


Fig. 2. 1 Chemical structure of amylose

In contrast, amylopectin is highly branched in structure, consisting of shorter α- (1, 4) linked chains of between 10 and 60 glucose units (Galliard and Bowler, 1987). These chains are connected to one another via α- (1, 6) linkages, as demonstrated in Fig. 2.1. The polymer's molecular weight is usually in excess of 108, 11making it one of the largest molecules in nature. Table 2.1 shows the comparative study of amylose and amylopectin.

Starch contributes greatly to the textural properties of many foods and is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent. The physicochemical properties and functional characteristics of starch systems and their uniqueness in various food products vary with starch biological origin. Native starch is a good texture stabilizer and regulator in food systems, but limitations such as low shear resistance, thermal resistance, thermal decomposition and high tendency towards retrogradation limit its use in some industrial food applications due to which some modifications are made in native starch to change its properties (Svegmark and Hermansson, 1993).

2.4 Physicochemical properties of starch

2.4.1 Gelatinization

Gelatinization is the process of granule swelling that breaks down the intermolecular bond of starch molecule in the presence of heat and heat allowing the hydrogen bonding site to engage more water followed by disruption of granule structure in which the loss of crystalline order can be observed in the disappearance of the X-ray diffraction.

According to Jane (2004), the temperature range at which starch granules lose their ordered structure in the presence of excess water is the gelatinization temperature. The gelatinization temperatures of sorghum starch vary from 71 to 80°C. Penetration of water increases randomness in the general structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water entry. Heat causes such regions to be diffused, so that the chains begin to separate into an amorphous form (Singh *et al.*, 2007). The granules/grains swell to 5 times their original size. Starch gelatinization ranges of different source are shown in Table 2.1.

Table 2.1 Amylose, amylopectin content and gelatinization temperature of different cereal starch.

Types of starch	Amylopectin (%)	Amylose (%)	Gelatinization range (°C)
Sorghum	73	27	71-80
Corn	73	27	62-72
Waxy corn	99	1	63-75
Buckwheat	77	23	67-75
Potato	78	22	58-67
Rice	83	17	62-78
Tapioca	82	18	57-65
Wheat	76	24	58-64

Source: Sarkar (2006)

2.4.2 Retro-gradation

During storage starch pastes may become cloudy and eventually deposit an insoluble white precipitate. This is caused by the re-crystallization of starch molecules; initially the amylose forms double helical chain segments followed by helix-helix aggregation(Sarkar, 2006).This phenomenon is termed retrogradation. Amylose is considered primarily responsible for the short-term retrogradation process due to the fact that the dissolved amylose molecules reorient in a parallel alignment. The long-term retrogradation is represented by the slow re-crystallization of the outer branches of amylopectin (Miles *et al.*, 1985). The re-crystallized amylopectin in the retrograded gel can be melted at 55°C, whereas for the re-crystallized amylose the melting temperature rises to 130°C.

In addition to the origin of starch, retrogradation also depends on starch concentration, storage temperature, pH, temperature procedure and the composition of the starch paste.

Retrogradation is generally stimulated by a high starch concentration, low storage temperature and pH values between 5 and 7. The salts of monovalent anions and captions can retard starch retrogradation (Swinkels, 1985a)

2.4.3 Solubility

Starch is not soluble in cold water. But its suspension starts to become viscous by heating and turns to transparent paste. It means amylopectin forms crystallized micelle and amylose arranges orderly around the gaps of the micelles in starch particle. That is why starch is not soluble in cold water. However, water molecules are getting into micelles gradually when heating the solution and resulting to loosen hydrogen bond and short molecules of amylose starts to dissolve, then amylopectin swells up.

2.4.4 Some properties required for film preparation

The water binding capacity is observed higher in the starch where amylose and amylopectin are loosely associated (Singh *et al.,* 2004). Similarly, oil absorption capacity is also important because of its role in storage stability and particularly in the rancidity development (Siddiq *et al.,* 2010). Likewise, bulk density is important for determining packaging requirements, material handling and application in wet processing in the food industry (Ocloo *et al.,* 2010). Also, dispersibility determines the tendency of flour to move apart from water molecules and reveals its hydrophobic action (Eke-Ejiofor *et al.,* 2014). Dispersibility is increased by starch gelatinization which increases the water-binding capacities (Dengate, 1984). Moreover, Hydrothermal treatment below the gelatinization temperature changes the physicochemical properties of starches without destroying the molecular and crystalline structure (Chen *et al.,* 2014). Similarly, acid alcohol modification might significantly affect physicochemical properties of starches by destroying the molecular and crystalline structure. Brunnschweiler *et al.,* (2006) reported that amylose aggregation has a strong impact on the texture of the pastes. Similarly, the un-swollen starch granules remain dense reflecting the maximum of light entering the medium (Achille *et al.,* 2007) and rendering less clarity in them. Likewise, coatings restrict gas exchange through peel of produce and thus lead to a modified internal atmosphere and an extended storage life of produce (Baldwin *et al.,* 2011). The rate of respiration was found to be decreased with the addition of plasticizers (Šuput *et al.,* 2013). There was decrease in rate of

respiration with addition of plasticizer as they improve integrity and avoid pores and cracks and thus promotes an effective barrier to gas exchange (García *et al.*, 1999). Due to acid alcohol modification of the starch, the amylose content decreases (Wang and Copeland, 2015). For the good quality of the film formation, amylose content should be high (Lourdin *et al.*, 1995). Interaction between glycerol starch molecular chain make them more reducing in terms of respiration (Sanyang et al., 2015a). The retention of nutritional factor such as vitamin, retinol depends upon the respiration rate of fruits and vegetable (Greenwood, 2019). One molecule of β carotene gives two molecules of vitamin A (Ophardt and Emeritus, 2019).

2.5 Starch extraction procedures

Common to all starch isolation procedures any immature or damaged potato tubers were removed and damaged part of tubers were initially cut away. Potato tubers were then rubbed or brushed in water to remove adhering dirt, surface infected skin and infected skin. Sodium bisulphate solution commonly used to inhibit browning was added in two of the processes i.e. Laboratory b and Pilot b. Starch isolation method, Laboratory a. Potatoes (1 kg) were washed carefully in tap water. The potatoes were cut in smaller pieces, macerated with added tap water in a blender equipped with razor blades and filtered through two layers of gauze. The filtrate was washed extensively with cold tap water, in order to separate starch granules and potato cell debris. The residual containing the starch grains were further washed by several cycles of centrifugation (2000g, 10 min) and air dried over night at room temperature. Starch isolation method, Laboratory b. Potatoes (1 kg) were washed carefully in tap water, dried and processed through a juice presser. The residual is filtrated through a sieve (mesh 125 mm) with addition of 1 L tap water removing cell wall material. To the residual starch slurry (final volume 2 L) is added 2 ml 38–40% sodium bisulphate solution and the slurry stands to settle for 1/2 h. The pellet of starch is washed two times in 1 L tap water and allowed to stand for 1/2 h. Finally, the starch is dried at room temperature on filter paper over night. Starch isolation method, small pilot plant scale, Pilot a. Potatoes (10 kg) were cleaned by an extensive wash in tap water. The potatoes were thereafter macerated in a Quadro Comill (model 194AS) by use of a series of sieves (meshes: 6350 mm, 812.8 mm, 475.2 mm and 228.6 mm) and rinsing with a total of 40 L of tap water. The starch was separated from the macerated potato slurries in a small hydro cyclone battery consisting of 14 hydro cyclones of which the 10 were blocked. The slurry was

passed over the hydro cyclones in three cycles after which the starch was concentrated by centrifugation and dried at room temperature over-night. Starch isolation method, medium pilot plant process, Pilot b. Potatoes (100 kg) were disintegrated with a grater and the rasping was collected on a 250 mm screen. Starch was flushed through the screen with distilled water until the starch stream ebbed away. Discoloring of the juice was hampered by an immediate addition of 20 ml, 1% bisulphite solution to the crude starch milk. A series of sequential sedimentation, suspension and sieving was performed in order to remove potato cell debris. Starch was allowed to sediment; the supernatant was decanted and distilled water (three times the sediment volume) was added. The starch was brought in suspension by stirring, passed through a 125 mm screen flushed with distilled water. The slurry was collected in an Inhofe cone, allowed to rest for 3–5 min after which the supernatant was decanted leaving 5–10% of the starch in the cone. The supernatant was filtered through a 75 mm screen. The filtrate was combined with the residual sediment of the cone and the sedimentation and wash was repeated twice. The final sediment was approximately 25 Baume´ (Be´), where Baume´ modulus 145: Be´ = 145 145/specific gravity at 60 °F (Cleland *et al.*, 1943). Concentrations of large starch slurries where achieved on a hydro cyclone battery to a concentration of not more than 21 Be´ in order to be able to pump the slurry. Starch isolation method, factory pilot plant process, Industry. Fresh potatoes were carefully cleaned before starch extraction. Soils were removed on rotating bar screens and adhering impurities were removed by intensive washing with tap water. High-speed ruptures open all cells in the potato tissue and the starch granules and juice were separated from cell walls on rotating conical screens using undiluted juice as flushing medium. The crude starch milk was washed on multi-stage hydro cyclones in counter current with tap water added to the last stage. The resulting purified starch milk had a concentration and viscosity of 22 Be´. Most of the water was removed on a rotating vacuum filter and the dewatered filter cake was dried in a stream of hot air in a flash dryer. The dried starch and drying air were separated on cyclones, the starch was cooled in a stream of cold air and the cooled dried starch was screened on centrifugal screens (source isolation of starch) (Wischmann *et al.*, 2007).

2.6 Modification of starch

The definition for modified starch is “Starch which has been treated physically or chemically to modify one or more of its key physical or chemical properties.” Starches or their derivatives can

be used in food as major ingredients or as an additive to optimize processing efficiency, product quality or shelf life. Starches are of various types; native starch, pre-gelatinized starch, cold-water swellable starch, retrograded starch and modified starches, which include, acetylated, cross-linked stabilized starches. Starch can be modified after isolation and added during preparation in modified form.

Native starches/ flour has a narrow peak viscosity. They become thick for a short time, and then begin to break down. They do not stand up well to processing, and produce a low quality final product. Starches are modified to prolong maximum viscosity. In particular, the properties of a starch product are not stable with respect to time and it is difficult to form long shelf-life food. To the extent possible, these inherent characteristics are explained by food processors to meet specific needs. Due to the sub-optical behaviour of native starch, modification of starch is the efficient way to provide starch products with suitable properties to meet the needs for specific uses (Shrestha, 2011).

2.6.1 Methods of starch modification

Starch modification is a process where the alteration of starch structure is by affecting the hydrogen bond in a controllable manner. Modification of starch can be carried out by chemical (Singh *et al.*, 2007) physical (Jane *et al.*, 1992) or enzymatic treatments (Kennedy *et al.*, 1987). The chemical modification is done by altering the hydroxyl group through chemical reaction such as esterification, etherification or oxidation. The starch can also be hydrolyzed by acid solutions (Singh *et al.*, 2007).

Chemically modified starches are of significant importance in many industrial applications. It can be used to improve functional properties of food products and used in the production of glue, coatings, chemicals and building materials. Some chemical methods were used to produce simple carbohydrates through hydrolysis, cross-linking or oxidation. Different modification procedures have been listed in Table 2.2

Table 2.2 Different starch modification types

Modifications	Types
Physical methods	Heat / Moisture treatment
	Pre-gelatinization
Conversion	Partial acid hydrolysis
	Partial enzymatic hydrolysis
	Alkali treatment
	Oxidation / blanching
Derivatization	Etherification
	Esterification
	Cross linking with phosphates

Source: Singh *et al.* (2007)

Acid hydrolysis has been used to modify starch for over 150 years. This process involves suspending starch in an aqueous solution of hydrochloric acid or sulphuric acid at certain temperatures. In the presence of a strong acid and heat, the glycosidic bond between monosaccharide in a polysaccharide is cleaved (Yiu *et al.*, 2008). Hydrolysis of starch by enzymatic method has some advantages over the acid or chemical method, especially if the hydrolyzed starch is intended for industrial application (Kennedy *et al.*, 1987). The enzymatic method produces a higher yield because the enzyme hydrolyzes a specific substrate; and fewer by-products are obtained, thus less purification is required.

The physically modified starch is usually made by applying a simultaneous action of temperature, pressure, shear and moisture. These physical treatments result in the modification of the granular structure at the molecular level.

2.6.1.1 Modification by hydrothermal treatment

Hydrothermal treatment (HTT) is a physical modification. The principal of this method is to heat the native starch at temperatures of 95-140°C, with limited moisture content (18- 27%) of the samples for 1-18 h (Blanshard, 1987). The temperature is increased at a rate of 2°C/min. This treatment would alter the structure, and thus would modify such properties as gelatinization and rheological behaviors of the starch, and certain desired functional properties would be attained. The treatment does not change the granular shape of the starch, but did alter the crystalline structure (Shrestha, 2011).

Collona *et al.* (1987) have reviewed some changes of the properties obtained from heat moisture treated starch. Some important changes include:

a) The gelatinization temperature is altered to broader and higher temperature ranges, compared to the native starch.
b) The reduction in swelling power. This is probably because the treatment would produce a hard shell of the granule (case-hardening), which is resistant to water absorption.
c) The increase in enzyme susceptibility. The degradation of starch granules has improved the enzymes' accessibility to starch.
d) A more viscous and a more stable paste of the treated starch.
e) Commercial heat-moisture treated corn starches have been developed by Japanese Sanwa Corn Starch Co. Ltd, who claimed that the products were economical and can improve the properties of food products such as texture, taste, stability, and thickening (Kudo, 1993).

The HTT may make the granules resistant to deformation by strengthening the intra granular binding force and it was speculated that in the annealed starch, swollen gelatinized granules were more rigid, contributing significantly to high cold viscosity.

2.6.1.2 Acid alcohol modification

All of the modified starches were readily soluble in hot water and their molecular weights decreased progressively from methanol modified starches to 1-butanol modified ones. The modified starches showed uniform granular appearance. Fox and Robyt (1992) continued their study on starches and investigated how acid concentration influences the hydrolysis inside the

granule. Their results confirm that the mechanism of hydrolysis of starch granule suspended in alcohol involves the hydrolysis of the glycosidic bonds with the water inside the granules (Fox and Robyt, 1992).

Acid hydrolysis of starch in alcohol has high recovery of starch and uses less amount of acid (Lin *et al.*, 2003). The average degree of polymerization of treated starch was affected by the botanical source and concentration of starch, type and concentration of alcohol, acid concentration and treatment temperature (Chang *et al.*, 2004; Fox and Robyt, 1992). The molecular weight distribution of acid-alcohol treated starch determined by high-performance size-exclusion chromatography (HPSEC) illustrated that the amylopectin fraction obviously decreased and shifted toward amylose fraction after treatment (Chang *et al.*, 2004; Lin *et al.*, 2003). The product was a relatively heterogeneous, high molecular weight starch. This method is used for preparing commercial "soluble starch".

2.7 Biodegradable starch films

There is an increasing interest in utilizing renewable resources as food packaging. The use of biodegradable polymers for packaging offers an alternative and partial solution to the problem of accumulation of solid waste composed of synthetic inert polymers. Usually, the film-forming substances are based on proteins, polysaccharides, lipids and resins or on a combination of these (Bertuzzi *et al.*, 2007b).

Polysaccharides such as starches, cellulose derivate and plant gums are being studied as edible films and coatings in food packaging and preservation (Baldwin *et al.*, 1995). Starch films and coatings have been used for various food and pharmaceutical applications. Edible films are viewed as an alternative for increasing the shelf life of fruits and vegetables, protecting them from the effects of humidity and oxygen, and thus delaying their deterioration.

Films prepared from starches are isotropic, odorless, tasteless, colorless, non-toxic and biodegradable. Edible films and coatings can be prepared from native and modified starches. The starch films have low oxygen permeability (Forsell *et al.*, 2002b). Starch coatings are nutritious, safe and economic and have been used in the storage and marketing of foods (Baldwin *et al.*, 1995). The mechanical properties like tensile strength (TS) and percentage

elongation of synthetic polymers such as low-density polyethylene (LDPE) and high-density polyethylene (HDPE) are significantly higher than those of the biopolymer films (Cunningham *et al.*, 2000b). However, the latter do have the potential to replace the conventional packaging in some applications. Although the water vapor transmission rate (WVTR) of biopolymers is higher than good barrier materials such as LDP, they are sufficient for short term (hours-days) protection against moisture.

The qualities of renewability, degradability, compost-ability, and edibility make such films particularly appealing for food and non-food packaging applications. Moreover, wide commercialization of biopolymer films will provide a value-added innovative use for traditional agricultural commodities as sources of film forming materials. Amylose is responsible for the film-forming capacity of starch-based films. High amylose induces strong gel network with water after gelatinization and is responsible for film forming capacity of starch-based films. It would result in strong and flexible films due to amylose crystallization (Bertuzzi *et al.*, 2007b).

2.8 Plasticizers

Starch films are usually modified by the addition of plasticizers to overcome the brittleness and make them more manageable. Polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers. Water also acts as a plasticizer in hydrophilic films. These plasticizers decrease the intermolecular attraction/forces between adjacent polymeric chains and increase the mobility of polymer chains, resulting in film flexibility and decrease in film strength (Laohakunjit and Noomhorm, 2004).

The plasticizing effect is based on the weakening of hydrogen bonds and the dipole– dipole intra and intermolecular interactions due to shielding of these attractive forces by the plasticizer molecules. As a consequence, free volume increases and glass transition temperature decreases, which significantly affect their mechanical properties. Palviainen *et al.* (2001) and Krogars *et al.* (2002) have also used native starch films and coatings for the preparation of tablets and pellets for the drug release. The alpha linkages of amylose starch allow it to be flexible and digestible. High starch content plastics are highly hydrophilic and readily disintegrate on contact with water. This can be overcome through blending with some plasticizer or use other chemical

to modify the starch, as the starch has free hydroxyl groups which readily undergo a number of reactions such as acetylation, esterification and etherification.

2.9 Film making processes

Most biopolymers are hydrophilic and, thus, water is the solvent used most often to dissolve biopolymers to obtain film forming solutions. Instead of water, some other solvents with or without water can be used to dissolve biopolymers. Usually, heating with solvent is needed to disrupt the native structure of the biopolymer to obtain a film forming solution. Plasticizer is added to the film forming solution at a convenient stage of the process to obtain flexible and elastic films which are often desired. There are various biomaterial film forming processes such as casting, spraying, extrusion and thermo-molding. The most common process to produce films on a laboratory scale is casting, which is used to produce free films for testing. In this process, a film forming solution is cast on a non-adhesive surface. Water or solvent is evaporated from the solution in order to form the film (Anker *et al.*, 2001; Lazaridou and Biliaderis, 2002; Rindlav *et al.*, 2002). As a result of solvent evaporation, biopolymer increases, with the result that hydrogen bonds are formed and basic film structure is created.

One application of casting is dipping, in which a product is dipped into the film forming solution to obtain a coating (Zevallos and Krochta, 2003). In the spraying process, a film forming solution is sprayed onto a surface of product on which droplets formed by a sprayer form uniform film. In spraying, solvent evaporates to some extent after leaving the nozzle of the sprayer allowing a shorter drying time for coating. Even if film formation occurs in a different way in casting and spraying processes the same starch-based film forming solution could be used in casting and spraying (Krogars *et al.*, 2002).

Continuous film forming can be carried out using extrusion which is widely used to produce synthetic polymer films. Extrusion has been used to produce films or sheets from starch wheat gluten (Hochstetter *et al.*, 2006) and mixtures of proteins and carbohydrates (Talja *et al.*, 2007). In thermo-molding, film forming materials, which are mixed with blender or extruder, are pressed between two heated plates to obtain films (Thunwall *et al.*, 2006).

A starch film forming solution is prepared by heating to gelatinize starch in excess water in which plasticizer is added before gelatinization (Mathew and Dufresne, 2002; Mehyar and Han, 2004) or after gelatinization into the hot solution (95°C) (Krogars *et al.*, 2002). In some studies film forming suspension containing native starch, amylose, amylopectin or mixture of amylose and amylopectin is heated in a pressurized vessel to complete amylose and amylopectin leaching into the solution (Mathew and Dufresne, 2002). After gelatinization, the film forming solution is poured onto a non-adhesive plate, such as polytetrafluoroethylene (teflon).

The longer the film formation takes, the longer time there is for a film component to phase separate and crystallize. Rindlav *et al.* (2002) have reported small and less aggregated amylose phases in the starch film for shorter drying times. Films prepared from starch or starch with added amylopectin resulted in a phase separated structure in the film. Moreover, structure of film prepared using starch with added amylose was more homogeneous, but crystallinity of films was higher than that of film produced from starch only (Rindlav *et al.*, 2002).

2.10 Respiration

Respiration is the chemical process by which fruits and vegetables convert sugars and oxygen into carbon dioxide, water, and heat. The heat generated by the respiration process tends to increase the temperature of a commodity. This, in turn, increases the water vapor pressure just below the surface of a commodity, leading to increased transpiration (Sastry *et al.*, 1977). Thus, it can be seen that respiration can cause transpiration to occur in saturated environments.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 2667\ KJ$$

The rate at which this chemical reaction takes place has been found to vary with the type and temperature of the commodity. More specifically, the rate of carbon dioxide production and heat generation due to respiration can be correlated to the temperature of the commodity. Coating on fruits represents the packaging material which act as the barrier for permeability of gases such O_2 and CO_2 and act as a modified Atmospheric storage (MAS). Low concentration of O_2 enhances the retention of vitamin because higher O_2 concentration leads to the higher respiration and higher production of heat and it may cause the depletion of nutritional parameters of fruits (Baldwin *et al.*, 1994).

2.11 Chlorophyll

Chlorophylls are unique pigments with green color and are found in diverse plants, algae, and cyanobacteria. The term chlorophyll is derived from the Greek chorus meaning “green” and phyllon meaning “leaf.” Isolation and naming of the chlorophyll was first carried out by Joseph Bienaimé Caventou (French pharmacist) and Pierre-Joseph Pelletier (French chemist) in 1817. Chlorophyll is made up of carbon and nitrogen atoms along with a magnesium ion in central position. Chlorophyll is found in almost every green part of plants, i.e. leaves and stem, within the chloroplast, the main organelle which contains the highest amount. Chloroplasts are found in the mesophyll layer, in the middle of plant leaves. Chloroplasts possess thylakoid membranes which contain green chlorophyll pigment. Chloroplast can be referred to as the “food factory” of the plant cell because it produces energy and glucose for the whole plant in association with CO_2, water, and sunlight.

The name “chlorophyll” was first given to the chloroplast of higher plants only, but later it was extended to all photosynthetic porphyrin pigments. It comes under the special class of compounds called tetra pyrrole because it contains four pyrrole rings joined together with a covalent bond, as are vitamin B_{12} and the heme molecule.

The main source of life on earth is the solar energy that is captured by green plants, algae, and various photosynthetic bacteria. Although there are different photosynthetic pigments such as carotenoids and phycobilins which entrap solar radiation, chlorophyll is the most important of these molecules. It converts solar energy into chemical energy that is used to build essential carbohydrate molecules (glucose) which are used as food source for the whole plant. The process can be described by the following equation:

$$CO_2 + \text{Water} \xrightarrow{\text{Sunlight and chlorophyll}} O_2 + \text{Glucose} + \text{Energy}$$

2.11.1 Types of chlorophyll

The numbers of naturally occurring chlorophylls may not yet be fully known. Chlorophyll a and chlorophyll b are the main components of photosystems in photosynthetic organisms. Initially, chlorophyll was classified into four –chlorophyll a, chlorophyll b, chlorophyll c, and chlorophyll

d but later a new type of chlorophyll was discovered within stromatolite (a hard rock structure made by cyanobacteria) in western Australia, which was named chlorophyll f. Thus, eventually chlorophyll was divided into five classes as a, b, c, d and f.

2.11.2 Chemistry of chlorophyll

The porphyrin unit has a very crucial role in nature because it participates in the fundamental skeleton of chlorophyll. Research has revealed that the chlorophylls are tetra pyrroles, a cyclic form of porphyrin and chlorine (the parent molecule of all chlorophylls). This cyclic form creates an iso-cyclic ring with the help of CH bridges. Chemically, chlorophylls possess a magnesium ion in the central position which is found connected with the tetra-pyrrole ring. Moreover, chlorophylls are hydrophobic molecules because they contain phytol, an esterified isoprenoid C_{20} alcohol. The phytol ($C_{20}H_{30}OH$) possesses a double bond in the trans configuration (Narashans *et al.*, 2017).

The presence of coating hinders respiration rate thereby preventing the substitution of Mg in chlorophyll molecule by hydrogen. This helps in prevention of change of chlorophyll into other pigments (Dea *et al.*, 2012). The structure of chlorophyll a has been given in Fig. 2.2.

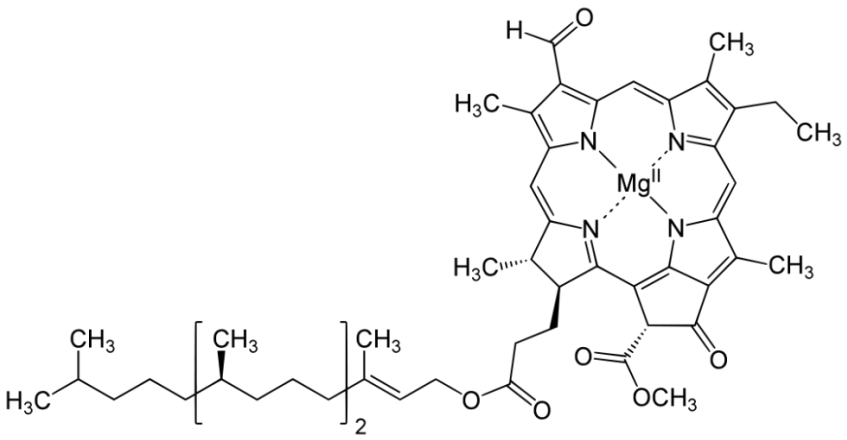


Fig. 2.2 Chemical structure of chlorophyll

Source**:** Joshna (2019)

2.12 Vitamin A

Vitamin A is a collective name for a group of lipophilic biomolecules required by humans to perform different vital metabolic functions. The vitamin exists in three major forms retinal (the aldehyde isoform), retinol (the alcohol isoform), and retinoic acid (RA), which is the irreversibly oxidized form of retinol. Retinal is the specific light-absorbing metabolite necessary for both types of vision i.e. scotopic (dim-light) vision and color vision. Retinoic acid (RA) is the active form of the retinol isoform of vitamin A, which functions as a hormone-like growth factor for epithelial cells and many other cell types of different tissues in the human body.

The significance of vitamin A is credited to the results of work dating back to 1906. It was first synthesized, however, in the laboratory in 1947 by two Dutch chemists, van Dorp and Arens.

β-carotene (BC), the red-orange pigment of carrots, is the major plant source of vitamin A precursor, and is represented as two connected retinyl groups. The molecule is utilized in the body where it contributes to the body's total vitamin A level. Some forms of carotenes such as α and γ also have some vitamin activity by virtue of each having a single retinyl group. Many other carotene isoforms existing in nature do not possess the biological activities of vitamin A.

The conversion of provitamin A into retinol is an actively regulated process. It seems that this conversion especially occurs in individuals who suffer from vitamin A deficiency. In 2001 the United States Institute of Medicine, recommended a unit called Retinol Activity Equivalent (RAE). According to this system, each µg RAE corresponds to 1 µg retinol, 2 µg of β-carotene in oil,12 µg of dietary β-carotene, or 24 µg of the three other dietary provitamin-A carotenoids.

In the absence of dietary fat-soluble vitamin, A, epithelial cells of the renal system can undergo typical changes that predispose to the formation of concretions (urinary calculi) in the urinary tract. Observations from classical animal studies showed that changes in the epithelial cells of mucous membranes can cause keratinization, such as xerophthalmia, and lengthening of the menstrual cycle. Earlier studies demonstrated a uniform degeneration of the medulla oblongata and other organs in animals deprived of dietary vitamin A (Oruch and Pryme, 2012).

Vitamin is directly related with light, O_2, moisture content and pH of produce. By the coating on produce, the coating serves as barrier to the moisture and light. Coating prevents direct contact with light by which vitamin depletion can be minimized. Coating can also act as a barrier to the moisture which can reduce the vitamin depletion (Barrett, 2018).

It has been extensively studied that fat-soluble Vitamin content of various vegetables are heat dependent. It has been explained by (Lešková *et al.*, 2006) that vegetables and fruits processed by applying heat treatments had reduced vitamin content than with commodities with no heat treatment. So, it can be inferred without a doubt that heat of respiration also results in similar phenomenon. This means that decrement of heat of respiration due to reduction in respiration rate (by biofilm coatings) ultimately increases vitamin retention. The structure of vitamin A has been shown in Fig. 2.3.

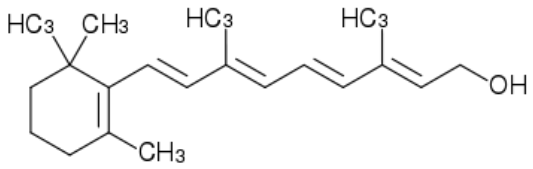


Fig. 2.3 structure of vitamin A

Source: Helmenstine (2017)

2.13 Retinol

Vitamin A-active compounds are represented by retinoids (designated as vitamin A) and their carotenoid precursors (provitamin A carotenoids). The retinoids comprise retinol, retinaldehyde, and retinoic acid, together with their naturally occurring and synthetic analogs.

Retinol derived from ingested provitamin A carotenoid, along with that ingested as such, is stored in the liver and secreted into the bloodstream when needed. The circulating retinol is taken up by target cells and converted in part to retinoic acid, which functions as a ligand to a nuclear retinoid receptor. The liganded receptor interacts with specific enhancer sites on the

DNA and, in collaboration with many other regulatory proteins, induces the synthesis of proteins through the direct control of gene transcription.

This type of action establishes vitamin A (in the form of the retinoic acid metabolite) as a hormone, similar to the steroid hormones and thyroid hormone. Vision is a nonhormonal, biochemical process involving a different vitamin A metabolite, 11-cis-retinaldehyde. The structures of retinoids found in foods and fish-liver oils are shown in Fig. 2.4.

The parent vitamin A compound, retinol, has the empirical formula $C_{20}H_{30}O$ and a molecular weight (MW) of 286.4. The molecule comprises a β-ionone (cyclohexenyl) ring attached at the carbon-6 (C-6) position to a polyene side chain whose four double bonds give rise to cis– trans (geometric) isomerism. Theory predicts the existence of 16 possible isomers of retinol, but most of these exhibit steric hindrance, and some are too labile to exist. The predominant isomer, all-trans-retinol, he liver and flesh of freshwater fish. Synthetic retinyl acetate ($C_{22}H_{32}O_2$) and retinyl palmitate ($C_{36}H_{60}O_2$) are used commercially to supplement the vitamin A content of foodstuff (Aung *et al.*, 1990). Structure of retinol has been shown in Fig. 2.4.

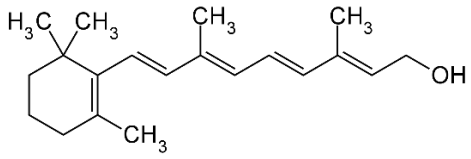


Fig. 2. 4 Structure of retinol

Source: Sorg *et al.* (2006)

2.14 Carotene

Carotenoids form one of the most important classes of plant pigments and play crucial role in defining the quality parameters of fruit and vegetables. They are found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts and molds, where they may carry out a protective function against damage by light and oxygen. The carotenoids are classified according to the structure.

1. The hydrocarbon carotenoids which are known as carotenes example β-carotene
2. The oxygenated carotenoids which are derivatives of these hydrocarbons known as xanthophylls (Saxena *et al.*, 2013).

Beta-carotene is a strongly colored red-orange pigment abundant in vegetables and fruits, especially in carrots and colorful vegetables. Beta-carotene is only manufactured in plants, not in humans and animals. In plants, beta-carotene absorbs light and energy, and is transferred to the chlorophyll for photosynthesis. The color fruits and vegetables have is due to the light that is not absorbed by the pigments and is reflected back to the environment. This is why carrots and other vegetables and fruits look the way they do - because beta- carotene reflects red orange and yellow light back into the eyes. There are many vegetables and fruits that contain beta-carotene; some of them are onions, broccoli, spinach, apricots, sweet potatoes, cantaloupes, pumpkins, and various herbs. As the name suggests, the name carotene is derived from the vegetable carrot, which in Latin is “carota”. Beta-carotene was named after carrots because the chemical was first discovered via crystallization of carrot roots in 1831. Wachenroder, the scientist who crystallized beta-carotene from carrot roots, came up with the name "carotene."

The chemical formula of beta-carotene is $C_{40}H_{56}$ and its structure was deduced by Paul Karrer in 1930. Beta-carotene is an organic compound and is classified as a hydrocarbon, specifically as a terpenoid. In addition, beta-carotene is a non-polar compound and is lipophilic, which means that is has the ability to dissolve in fats, oils, lipids, and other non-polar solvents. Its molar mass is 536.87 g/mol, has a density of 0.94 g/cm^3 , melting point of180℃, and a boiling point of 633 to 677℃ (Park, 2019). The structure of β-carotene has been shown in Fig. 2.5.

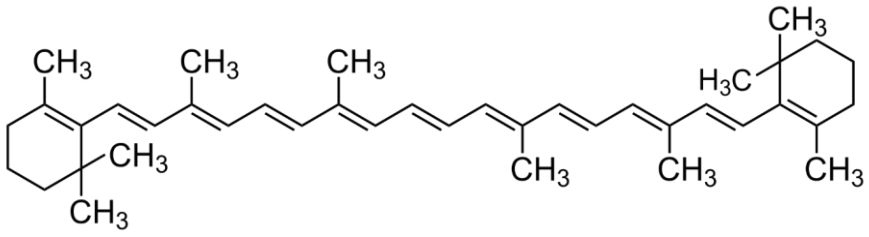


Fig. 2. 5 Structure of β-carotene

Source: Mezzomo and Ferreira (2016)

Part III

Materials and methods

3.1 Raw material collection

The raw materials for the research were obtained from the different haat-bazars of Dharan. The raw materials collected were waste potatoes and their peel.

3.2 Starch extraction and purification

The starch from potato waste and its peels were extracted and purified as described by Marshall (1969) with slight modification as described in Fig. 3.1.

3.3 Starch modifications

The extracted starch were modified by various treatment methods which are explained in sections 3.3.1 to 3.3.2.

3.3.1 Hydrothermal treatment

The extracted starch (100 g) were adjusted from 25% to 28% moisture, pH 6.7, and equilibrated at 4°C to 6°C overnight (refrigerated condition) and placed in a hot air oven for 3h at 110°C. The sample were shaken occasionally for even distribution of heat. The sample were cooled to room temperature (about 30°C) and dried at 50°C, equilibrated for 4h and sealed in polyethylene bags until use as described by Collado *et al.* (2001). The flowsheet for extraction and purification of starch is shown in Fig. 3.1.

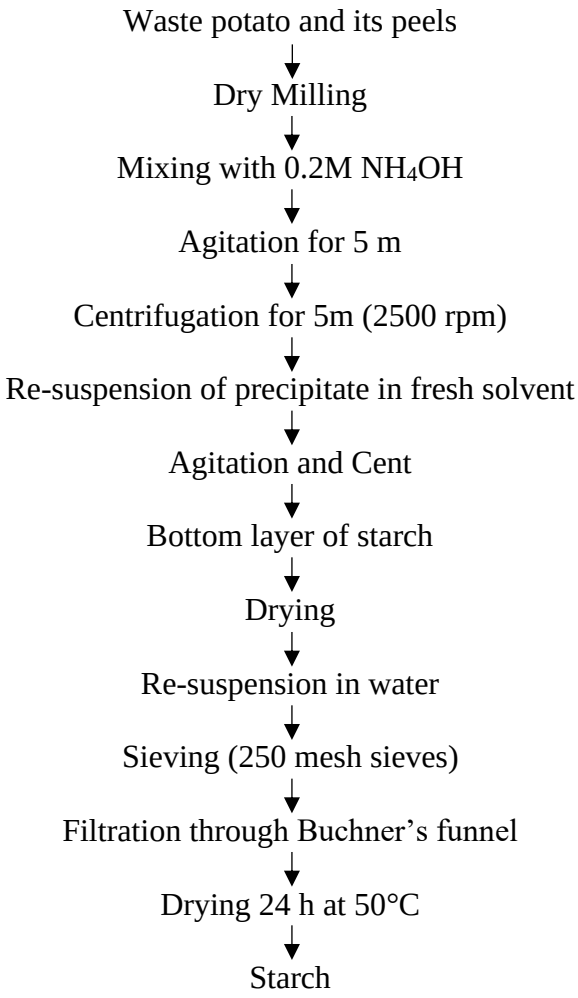


Fig. 3. 1 Extraction and purification of starch

3.3.2 Acid-alcohol modification

A sample of 25 g were suspended in 100 ml of ethanol in a 500 ml conical flask. The hydrolysis reaction was initiated by adding 36% hydrochloric acid (20 ml) and allowed to proceed for 1 h at 45°C in a water bath. The reaction was stopped by neutralizing the solution with 1M NaOH. The samples were then transferred into 50 ml centrifuge tubes and centrifuged at 3,500 rpm for 5 m. The supernatant was collected and the precipitate was held with 50% ethanol until neutral to litmus. The starch samples were filtered using Whatman no.1 filter paper and dried in an oven at 50°C. They were weighed at room temperature as described by Chang *et al.* (2006).

3.4 Physio-chemical characteristics of control, extracted and modified starch

The different physio-chemical characteristics of raw and modified starch was studied.

3.4.1 Iodine affinity of starch (IAS)

The iodine affinity of starch of flours was assayed using guidelines of Kawabata *et al.* (1984). 3 g each of flour were introduced into 50 ml beakers and made up to 30 ml dispersions using distilled water. The dispersion was stirred occasionally within the first 30m and then filtered through Whatman no. 42 filter paper. A 10 ml aliquot of the filtrate was pipetted into a conical flash, phenolphthalein was added and the filtrate titrated with 0.1N I_2 solution to a bluish back end-point. The starch cell damage (free starch content) was calculated using the titre value and expressed as iodine affinity of starch.

$$IAS\ (ppm) = \frac{VD \times V_t \times N_a}{V_A \times M_s \times 100} \times 10^6$$

Where IAS= Iodine Affinity of Starch; VD = Total volume of dispersion; V_A = Volume of aliquot used titration, V_t = Titer value, M_s = Mass (db) of flour used, N_a = Normality of iodine solution used

3.4.2 Flour dispersibility (D)

The flour dispersibility was determined by the method described by Kulkarni *et al.* (1991) . 10 g of flour were weighed into 100 ml measuring cylinder and distilled water added to make a volume of 100 ml. The set up was stirred vigorously for 1min. The volume of the settled particles was registered after regular time step of 30 m. The volume of settled particles was subtracted from 100. The difference was reported as percentage of dispensability.

3.4.3 Paste clarity (PC)

The paste clarity was determined according to the method of Craig *et al.* (1989). 1% aqueous suspension was made by suspending 0.2 g of flour in 20 ml of distilled water in a stoppered centrifuge tube and vortex mixed. The suspension was heated in a boiling water (100°C) bath

for 30 m. After cooling, clarity of the flour was determined by measuring percent transmittance at 650 nm against water blank on a spectrophotometer.

3.4.4 Oil absorption capacity (OAC)

The oil capacity of flour was evaluated according to Sosulski *et al.* (1976). 1 g of sample (M_o) was mixed with 10 ml in a weighed 20 ml centrifuge tube. The slurry was agitated on a vortex mixer for 2 m, allowed to stand at 28°C for 30 m and then centrifuged at 4500 rpm for 30 m. The clear supernatant was decanted and discarded. The adhering drops of oil were removed and the tube was weighted (M1). The AOC was calculated as follows:

$$OAC = \frac{M_1 - M_0}{M_0} \times 100$$

3.4.5 Water binding capacity

Water binding capacity (WBC) of the sample starch was determined using the method described by Yamazaki (1953) and modified by Medcalf and Gilles (1965). A suspension of 5 g starch (dry weight) in 75 ml distilled water was agitated for 1 h and centrifuged at 3000 rpm for 10 min. The free water was removed from the wet starch, which was then drained for 10 m.

3.4.6 Bulk density (BD)

The method described by Narayana and Narasinga (1982) was used for the determination of bulk density. 50 g of flour was put into 100 ml measuring cylinder. The measuring cylinder was then tapped continuously on a laboratory table until a constant volume was obtained. BD (g/cm^3) was calculated using following the formula:

$$bulk\ density a(g/cm\ ) = \frac{weight\ sample}{volume\ of\ sample\ after\ taping}$$

3.5 Preparation of starch films

The films of three types of dry starches (extracted, modified and pure commercial starch) were prepared according to the method described by Muller *et al.* (2008) with minor modifications. The films were prepared through the casting technique using a film-forming solution containing

5% of three types of starch. Three plasticizers (glycerol, sorbitol and polyethylene glycol) of different concentrations (35%, 45% and 55%) were used taking per 5 g of each dry starch. The mixture was heated to boiling temperature on a hot plate and constant stirring was done for 10 min by a magnetic stirrer. The mixture was cooled till bubbles vanish and poured (hot) about 45 ml homogenously onto the non-sticky plastic trays of diameter 13 cm. The trays containing the film forming solution was then dried in a cabinet dryer at 50°C for 5 h. The dried films were peeled off from the trays and kept in airtight polyethylene bags.

Table 3.1 Preparation of different starch film using different plasticizers for extracted starch.

Plasticizers	Concentration of Plasticizers	Extracted Starch	Hydrothermal treated starch	Acid-alcohol treated starches
glycerol	35%	√	√	√
	45%	√	√	√
	55%	√	√	√
Sorbitol	35%	√	√	√
	45%	√	√	√
	55%	√	√	√

3.6 Determination of rate of respiration

Rate of respiration is determined by measuring the CO_2 produced by the cultivar by titration as explained by Han *et al.* (2011). *Chaenomeles japonica* (Maule's quince) harvested in the period of its physiological maturity was collected and stored at room temperature and RH (33°C at RH 60%).

First of all, apparatus was set up as shown in Fig. 3.2. In the first chamber, there is solution of KOH. When air enters to this solution, KOH absorbs CO_2 present in air (Smirnova *et al.*, 2014). Now, after absorption of CO_2, the air which has no CO_2, passes to another chamber where the respiring surface (here, fruit in this case) respires using O_2 present in remaining air. This O_2 is consumed by the surface to release CO_2. Now, air with this CO_2 passes to another chamber where lime water $Ca(OH)_2$ is kept. On reacting with lime water, $CaCO_3$ precipitate is formed (Han *et al.*, 2011). The concentration of this precipitate can be measured by titrating with 0.1 N HCl.

The simple equation can be deployed to calculate rate of respiration, $R = k.x$, whereby, R= rate of respiration (volume O_2/unit time), k= rate constant and x = rate of $CaCO_3$ formation. The apparatus is shown in Fig. 3.2.

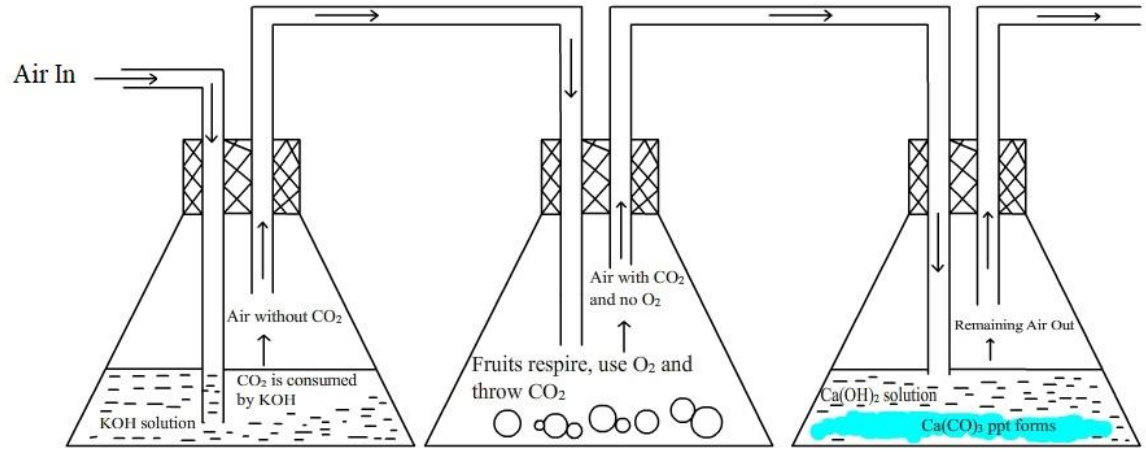


Fig. 3. 2 Apparatus for measurement of rate of respiration

3.7 Chlorophyll determination

Vegetables samples were prepared with a laboratory by grinding about 1 g fresh peel of *japonica*. Extraction was done by 90% aqueous methanol solution. Mixture was well mixed by using magnetic starrier for 1 min at 300 rpm.

The analytical determination was performed with spectrophotometer at the following wavelengths: 666 nm and 653 nm, for chlorophyll A and chlorophyll B and 470 nm for carotene.

Equations used for calculation were given by Costache *et al.* (2012).

Chlorophyll a = 15.65 ×666 A – 7.340 ×653 A

Chlorophyll b = 27.05 ×653 A – 11.21 ×666 A

3.8 β-Carotene determination

Carotene is a function of chlorophyll A and chlorophyll B. Carotene can be determined from the data of chlorophyll obtained. The formula for the determination of carotene from chlorophyll was taken from Costache *et al.* (2012).

Carotene = 1000 ×470 A – 2.860× Chlorophyll a – 129.2× Chlorophyll b/245

3.9 Vitamin A determination

Vitamin A is the function of carotene. Vitamin A can be determined from carotene. The relation between Vitamin A and carotene has been given by Casas (2007).

1 IU of vitamin A is biologically equivalent to 0.6 μg of β-carotene.

1 μg of carotene = 1/0.6 IU of vitamin A = 1.66 IU of Vitamin A.

3.10 Retinol determination

Retinol is the preformed vitamin A. Changes of vitamin A and Retinol are proportional to each other. The relation between vitamin A and Retinol is given below and can be calculated from following relation.

1 μg of retinol is equivalent to 3.33 μg of vitamin A

1 μg of retinol=1 μg of vitamin A /0.3

3.11 Statistical analysis

All the data obtained in this research work were analyzed by the statistical software SPSS, Discovery edition 3 (2008) and GenStat 12th edition. From this ANOVA (no blocking at 5% level of significance), Least Significant Difference (LSD) and mean were obtained to determine whether the samples were significantly different from each other and also to determine which one is superior to them.

Part IV

Results and discussions

4.1 Physicochemical properties of potato-starches Potato starch isolated from waste potatoes of different *haat-bazars*, hotels and restaurants of Dharan sub-metropolitan city were modified by two techniques viz. hydrothermal treatment (moisture adjusted to 28% and heated at 110°C for 3 h) and acid-alcohol treatment (treated with 100 ml of rectified alcohol and 20 ml of conc. HCl) to study the different physicochemical properties of the extracted starch such as oil absorption capacity, dispersibility, iodine affinity of starch, paste clarity, water binding capacity and bulk density. Functional properties of differently treated starches have been shown in Table 4.1 and Table 4.2. The values after '±' represents standard deviation; and the superscripts with different alphabets represent the values that are significantly different from each other.

Table 4.1 Functional properties (a) of differently treated starch

Treatments	Functional properties of differently treated Starch		
	Oil absorption Capacity (%)	Water binding Capacity (%)	Dispersibility (%)
Raw	230 ± 4^{c}	239.4 ± 5.04^{b}	51 ± 3.6^{c}
HTT	174.67 ± 4.5^{b}	277 ± 4.58^{c}	69 ± 2.65^{b}
AAT	136 ± 3.6^{a}	191.13 ± 6.4^{a}	60 ± 1^{a}

In the table, the values after '±' represent standard deviation ($p<0.05$) and the superscripts with different alphabets represent the values that are significantly different from each other.

Table 4.2 Functional properties (b) of differently treated starch

Treatments	Functional properties of differently treated Starch		
	Iodine affinity of starch (ppm)	Bulk Density (g/cm^3)	Paste clarity (%T)
Raw	498.33 ± 7.63^b	0.73 ± 0.01^a	29.67 ± 1.52^b
HTT	496 ± 1^b	0.825 ± 0.1^a	19.06 ± 1^a
AAT	320 ± 5^a	0.74 ± 0.085^a	$18 \quad 1^a$

In the table, the values after '±' represent standard deviation ($p<0.05$) and the superscripts with different alphabets represent the values that are significantly different from each other.

4.1.1 Water binding capacity

Water binding capacity (WBC) of extracted potato starch was 239.4% whereas for modified starches WBC varied from 191.1% to 277 %. Acid alcohol (AATS) modification decreased the water binding capacity to 191.1%. However, hydrothermal treatment (HTT) increased the water binding capacity to 277%. There was a significant difference ($P<0.05$) in water binding capacity between different modified samples when compared to extracted potato starch.

The drastic increase in the water binding capacity of HTT samples increased which might be due to that hydrothermal treatment (HTT) increases the hydrophilic tendency of starches. Similar results were explained by (Abraham, 1993). The differences in degrees of availability of water binding sites among the starches may have also contributed to variation in water binding capacity. The water binding capacity is observed higher in the starch where amylose and amylopectin are loosely associated (Singh *et al.*, 2004). Acid alcohol modification basically reduces water binding capacities because of reduction of the amorphous region in the starch granules. This reduces the number of available binding sites for water in the starch granule.

4.1.2 Oil absorption capacity

The OAC is the ability to absorb or retain oil. They are also important because of their storage stability and particularly in the rancidity development (Siddiq *et al.*, 2010). Oil absorption capacity of extracted starch was found to be 230 % whereas for modified starch the mean oil absorption capacity was 174.67% and 136 % for hydrothermal treated (HTT) and acid-alcohol treated (AAT) starches respectively for the *sunflower oil*. There was a significant difference ($P<0.05$) in oil absorption capacity between different modified samples when compared to extracted potato starch.

The OAC decreasing with starch modification could be attributed to a decreasing in protein due to hydrothermal treatment and acid alcohol modification which tend to reduce the hydrophobicity and thereby causing a low-fat binding to protein. The flour in this present study is potentially useful in structural interaction in food specially in flavor retention, improvement of palatability and extension of shelf life particularly in bakery or meat products where oil absorption is desired.

4.1.3 Bulk density

Bulk density is a property of powders, granules and other divided solids, especially used in references to mineral components, chemical substances, food stuffs and or any other masses of particulate matter. It is defined as the mass of many particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume, and internal pore volume. The bulk density is a measure of the heaviness of flours sample. It is important for determining packaging requirements, material handling and application in wet processing in the food industry (Ocloo *et al.*, 2010). Starch is attractive, low-cost filler for biodegradable polymers. When used in its native granular form, starch content in composite materials is usually limited by the loss of mechanical properties (compared with the unfilled polymer) to volume fractions ~0.30.

Bulk density of extracted starch was found to be 0.73 whereas for modified starch the mean bulk density was 0.825 and 0.74 for hydrothermal treated (HTT) and acid-alcohol treated (AAT)

starches respectively. There was a not significant difference (P>0.05) in bulk density between different modified samples when compared to extracted potato starch.

4.1.4 Dispersibility

The dispersibility is a measure of reconstitution of flour water. The dispersibility determines the tendency of flour to move apart from water molecules and reveals its hydrophobic action (Eke-Ejiofor *et al.*, 2014). Dispersibility of extracted starch was found to be 51 % whereas for modified starch the mean bulk density was 69% and 60% for hydrothermal treated (HTT) and acid-alcohol treated (AAT) starches respectively. There was significant difference (P<0.05) in dispersibility between different modified samples when compared to extracted potato starch (Kulkarni *et al.*, 1991) reported that the higher the dispersibility, the better the starch reconstitutes in water to give a fine and consistent paste. The increasing dispersibility of flour raw starch to HTT starch could be caused by starch gelatinization which increases the water-binding capacities (Dengate, 1984).

4.1.5 Iodine affinity of starch

The iodine affinity of extracted starch was found to be 498.33 ppm whereas for modified starch was 496 ppm and 320 ppm for hydrothermal treated (HTT) and acid-alcohol treated (AAT) starches respectively. There was significant difference (P<0.05) in the iodine affinity of starch between AAT starch when compared to extracted potato starch though HTT starch and extracted raw starches were not significantly different.

Hydrothermal treatment below the gelatinization temperature changes the physicochemical properties of starches without destroying the molecular and crystalline structure (Chen *et al.*, 2014). Similarly acid alcohol modification might have significantly affected physicochemical properties of starches by destroying the molecular and crystalline structure Brunnschweiler *et al.* (2006) reported that amylose aggregation has a strong impact on the texture of the pastes.

4.1.6 Paste clarity

Paste clarity of extracted starch was found to be 29.67% T whereas for modified starch the mean bulk density was 19.06 % T and 18 % T for hydrothermal treated (HTT) and acid-alcohol treated (AAT) starches respectively. There was significant difference ($P<0.05$) in paste clarity between raw starch with modified starches but there is no significant between HTT starch and AAT starch.

The paste clarity is an important that governs different applications of flours and starches for food processing. The low clarity of the HTT starch would be explained by the fact that the not swollen starch granules remained dense reflecting the maximum of light entering the medium (Achille *et al.*, 2007). HTT starch had minimum swelling power which might be due to production of hard shell of granule (case hardening) during hydrothermal treatment which is resistant to water adsorption which helped in lower transparency. Pastes obtained from AAT starch were less transparent than raw starch suspension m HTT starch had minimum swelling power. This may be due to production of hard shell of granule (case hardening) during hydrothermal treatment which is resistant to water adsorption (Achille *et al.*, 2007).

4.2 Yield of starch films

Extracted starch from the potato waste was modified by different techniques namely hydrothermal treatment (moisture content adjusted to 28% and heated at 110°C for 3 h and acid-alcohol treatment (treated with 100 ml of alcohol and 20 ml of conc. HCl). Modified as well as extracted starches with varied glycerol and sorbitol concentration (35%, 45%, 55%) were used for the preparation for starch-based films. Starch based films were analyzed for its yield from 5 g of starch and its thickness and diameter.

The yield of the starch was 7.5±1% from the waste potatoes from the different place of Dharan. Four to five films (moisture content 10±2 %) of 1.5±0.45 g were produced from 5 g starch of thickness 125±8.5 microns and diameter of 9 cm. The yield of the starch might have varied due to the variation of potatoes types and various extents of deteriorations. The yield of starch films might have varied due to the variations in thickness. The thickness varied due to inconsistent pouring in the petri-plate while forming the films.

4.3 Rate of respiration with starch coat in *Chaenomeles japonica*

Extracted starch from the potato waste was modified by different techniques namely hydrothermal treatment (moisture content adjusted to 28% and heated at 110°C for 3 h.) and acid-alcohol treatment (treated with 100 ml of alcohol and 20 ml of conc. HCl). Modified as well as extracted starches with varied glycerol concentration (35%, 45%, 55%) were used for the preparation for starch-based suspension. *Chaenomeles japonica* (Maule's quince) was dipped in the suspension so that the coat was uniform. The rate of respiration was measured up to 3 days at 33°C at RH 60%.

Respiration rate of coated and control fruits continuously increased during storage time and shelf life condition; however, fruits respiration rate was significantly affected by coating treatments. Rate of respiration (mg CO_2/kg/h) for *Chaenomeles japonica* (Maule's quince) was determined for 3 respective days by uniform coating with different forms of starches treated with glycerol and sorbitol at different concentration. The result of the rate of respiration at 33°C at RH 60% is represented in Fig. 4.1.

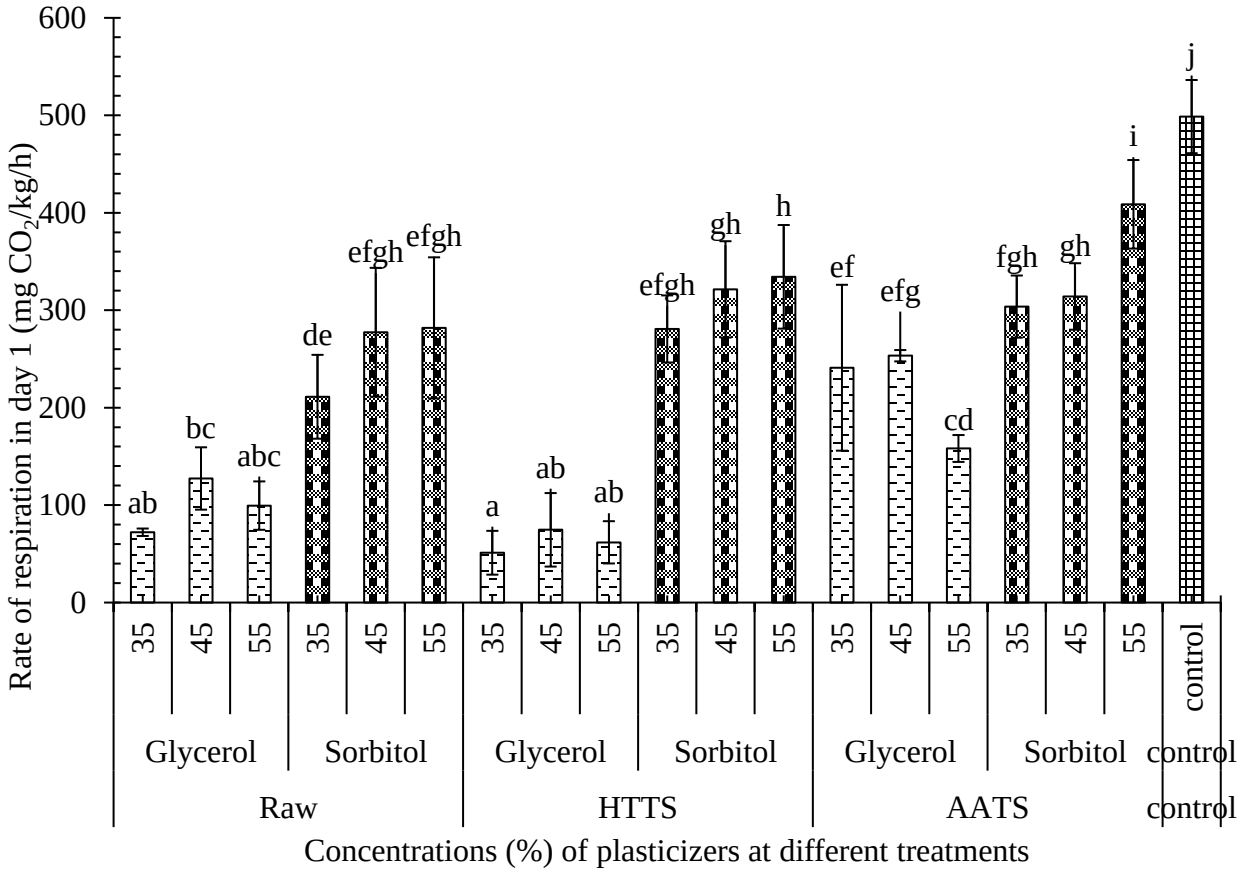


Fig. 4.1 Rate of respiration of coated *Chaenomeles japonica* in day 1

From Fig. 4.1, it was found that the respiration rate has maximum value (408.7 mg CO_2/kg/h) in ATTS treated starch plasticized with 55% sorbitol and minimum value (51.1 mg CO_2/kg/h) in HTTS treated starch plasticized with 35% glycerol when compared with the uncoated sample (498.7 mg CO_2/kg/h). There is significant difference observed between these treatments.

The respiration rate of *japonica* fruit in day 1, when 35% ,45% and 55% of the glycerol used as a plasticizer in the starch coat there was no significance difference in HTTS and extracted potato starch paste but significant difference with AATS coat. When 35% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS and AATS but significant difference with extracted potato starch paste coat. When 45% and 55% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS, AATS and

extracted potato starch paste coat. From the above results glycerol treatment seems to be more effective than sorbitol treatment.

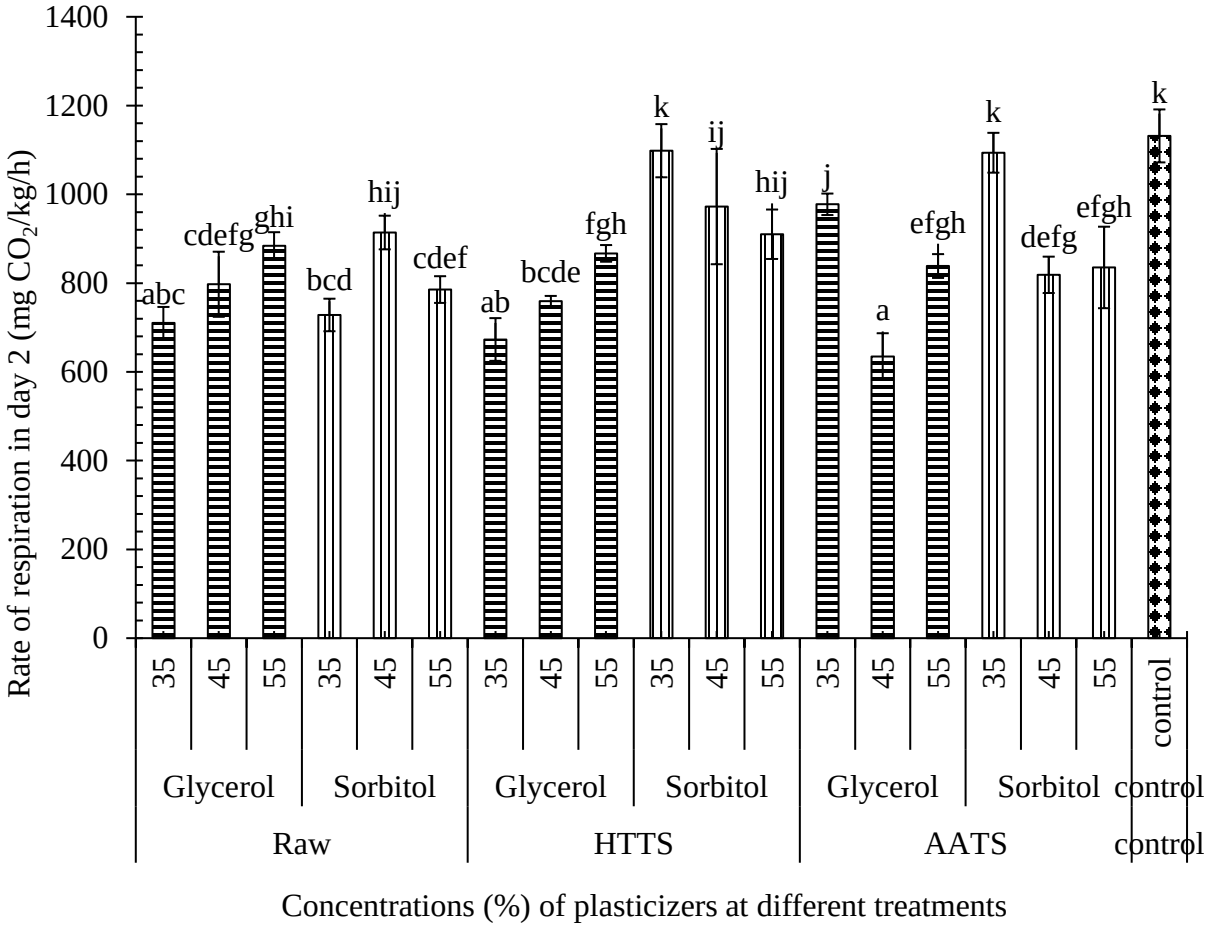


Fig. 4. 2 Rate of respiration of coated *Chaenomeles japonica* in day 2

From Fig. 4.2, it was found that the respiration rate has maximum value (1098.3 mg CO_2/kg/h) in HTTS treated starch plasticized with 35% sorbitol and minimum value (634.5 mg CO_2/kg/h) in AATS treated starch plasticized with 45% glycerol when compared with the uncoated sample (1131.7 mg CO_2/kg/h). There is significant difference observed between these treatments.

The respiration rate of *japonica* fruit in day 2, when 35% of the glycerol used as a plasticizer in the starch coat there was no significance difference in HTTS and extracted potato starch paste but significant difference with AATS coat. When 45% of the glycerol used as a plasticizer in

the starch coat there was no significance difference in HTTS and extracted potato starch but significant difference with AATS paste coat. When 55% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS, AATS and extracted potato starch paste coat. when 35% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS and AATS but significant difference with extracted potato starch paste. When 45% and 55% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS, AATS and extracted potato starch paste coat. From the above results glycerol treatment seems to be more effective than sorbitol treatment.

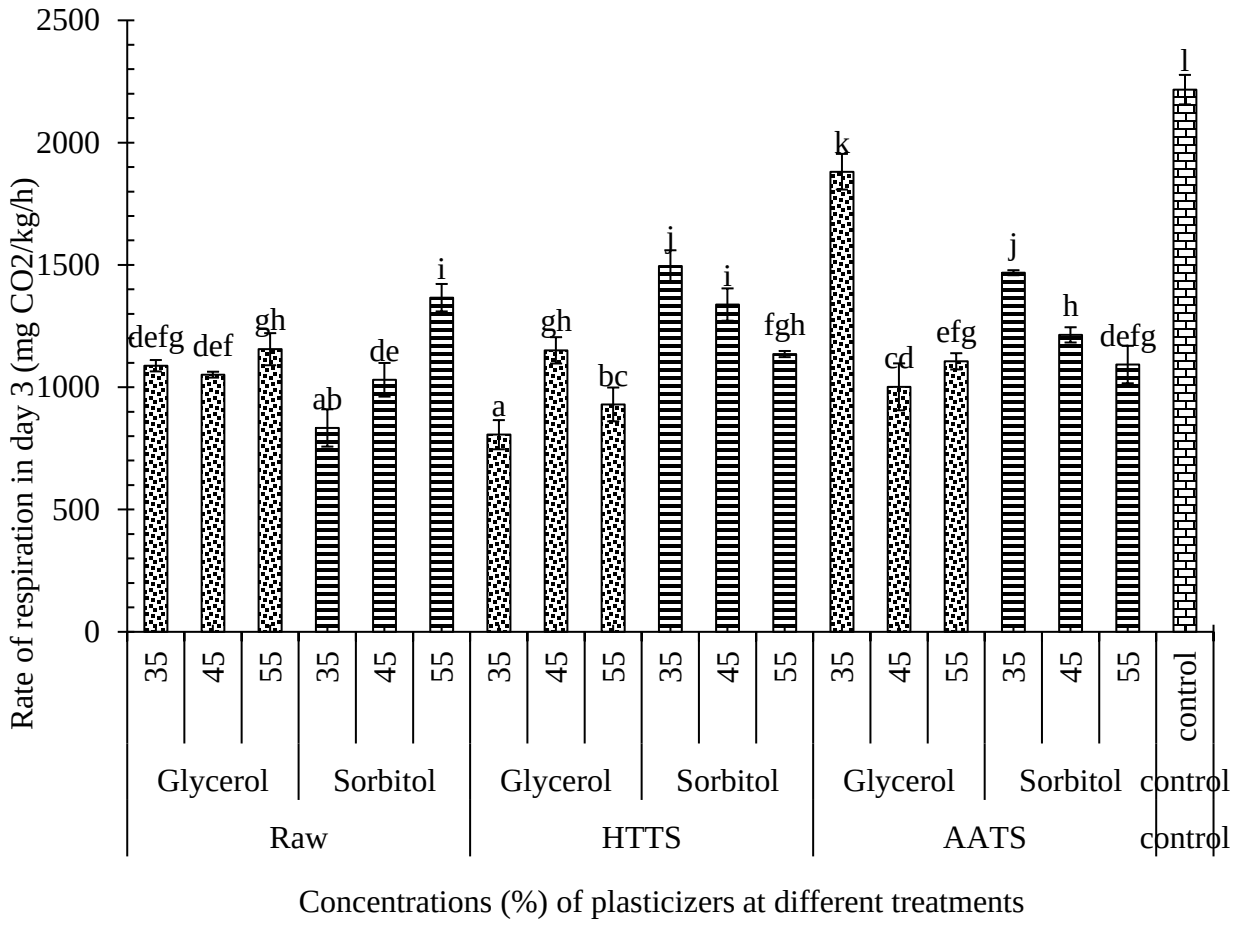


Fig. 4. 3 Rate of respiration of coated *Chaenomeles japonica* in day 3

In Fig. 4.3, it was found that the respiration rate has minimum value (806 mg CO_2/kg/h) in HTTS treated starch plasticized with 35% glycerol and maximum value (1881 mg CO_2/kg/h) in

AATS treated starch plasticized with 35% glycerol when compared with the uncoated sample (2217 mg CO_2/kg/h). There is significant difference observed between these treatments.

The respiration rate of *japonica* fruit in day 3, when 35% of the glycerol used as a plasticizer in the starch coat there was significance difference in all treated starch coats. When 45% of the glycerol used as a plasticizer in the starch coat there was no significance difference in all treated starch coats. When 55% of the glycerol used as a plasticizer in the starch coat there was no significance difference in AATS and extracted potato starch paste coat but significant difference with HTTS coat. When 35%, 45% and 55% of the sorbitol used as a plasticizer in the starch coat there was no significance difference in HTTS and AATS but significant difference with extracted potato starch paste. From the above results glycerol treatment seems to be more effective than sorbitol treatment.

The value of rate of respiration for non-coated fruit in day 1 and day 3 are 498.6667 and 2216.667 mg CO_2/kg/h. This means there was 344.5187% increase in the rate of respiration. The rate of respiration for uniform coated raw starch treated with 35% glycerol in day 1 and day 3 was 72.134 and 1087.986 mg CO_2/kg/h. This means that there was 1408.278% increase in the rate of respiration. The rate of respiration for uniform coated raw starch treated with 45% glycerol in day 1 and day 3 was 127.3211 and 1051.33 mg CO_2/kg/h. This means that there was 725.7337% increase in the rate of respiration. The rate of respiration for uniform coated raw starch treated with 55% glycerol in day 1 and day 3 was 99.47847 and 1155.562 mg CO_2/kg/h. This means that there was 1061.62% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 35% glycerol in day 1 and day 3 was 211.1563 and 833.590 mg CO_2/kg/h. This means that there was 294.7741% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 45% glycerol in day 1 and day 3 was 277.3528 and 1030.434 mg CO_2/kg/h. This means that there was 271.5428% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 55% glycerol in day 1 and day 3 was 281.8412 and 1366 mg CO_2/kg/h. This means that there was 384.6701% increase in the rate of respiration. The rate of respiration for uniform coated AATS starch treated with 35% glycerol in day 1 and day 3 was 51.09856 and 805.9856 mg CO_2/kg/h. This means that there was 1477.316% increase in the rate of respiration. The rate of respiration for uniform coated AATS starch treated with 45% glycerol in day 1 and day 3 was

74.63884 and 1150.859 mg CO_2/kg/h. This means that there was 1441.903% increase in the rate of respiration. The rate of respiration for uniform coated AATS starch treated with 55% glycerol in day 1 and day 3 was 61.80374and 929.7217 mg CO_2/kg/h. This means that there was 1404.313% increase in the rate of respiration. The rate of respiration for uniform coated raw starch treated with 45% sorbitol in day 1 and day 3 was 321.3905 and 1338 mg CO_2/kg/h. This means that there was 316.316% increase in the rate of respiration. The rate of respiration for uniform coated raw starch treated with 55% sorbitol in day 1 and day 3 was 334.2541and 1135.587 mg CO_2/kg/h. This means that there was 239.7376% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 35% sorbitol in day 1 and day 3 was 240.9598 and 1881.128 mg CO_2/kg/h. This means that there was 680.681% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 45% sorbitol in day 1 and day 3 was 253.2965 and 1001.584 mg CO_2/kg/h. This means that there was 295.4193% increase in the rate of respiration. The rate of respiration for uniform coated HTTS starch treated with 55% sorbitol in day 1 and day 3 was 158.1052 and 1106.001 mg CO_2/kg/h. This means that there was 599.5346% increase in the rate of respiration. The rate of respiration for uniform coated ATTS starch treated with 35% sorbitol in day 1 and day 3 was 303.7022 and 1468.201 mg CO_2/kg/h. This means that there was 383.4345% increase in the rate of respiration. The rate of respiration for uniform coated ATTS starch treated with 45% sorbitol in day 1 and day 3 was 314.0906 and 1214.039 mg CO_2/kg/h. This means that there was 286.5251% increase in the rate of respiration. The rate of respiration for uniform coated ATTS starch treated with 55% sorbitol in day 1 and day 3 was 408.6667and 1092.791 mg CO_2/kg/h. This means that there was 167.404% increase in the rate of respiration.

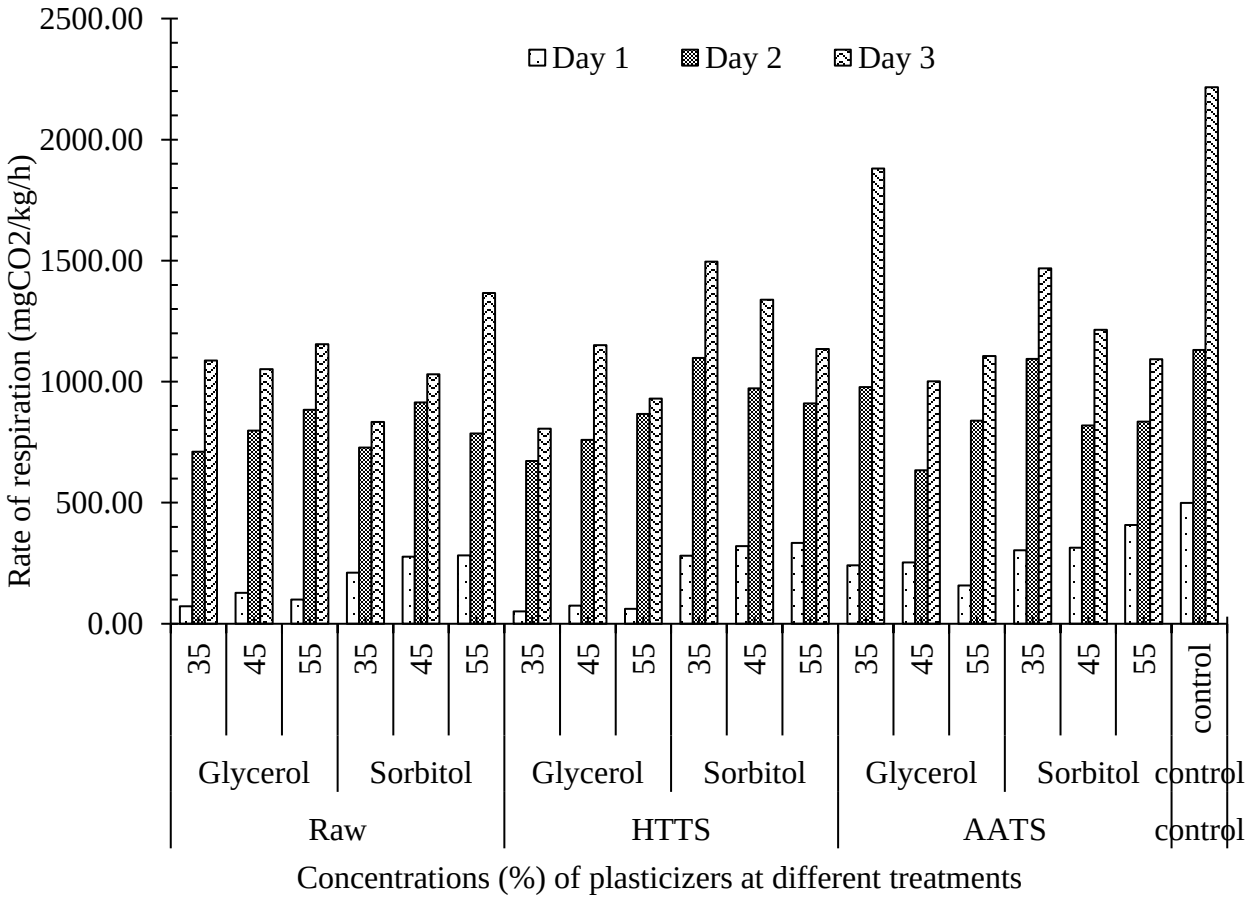


Fig. 4.4 Comparison of the rate of respiration of the coated *Chaenomeles japonica* in different days

The coating has improved the quality of *japonica* fruit. The rate of respiration was found to be decreased in the coated fruit in compared to non- coated fruit. Coatings can extend storage life of produce by a similar mechanism as CA and MAS. Coatings restrict gas exchange through peel of produce and thus lead to a modified internal atmosphere and an extended storage life of produce (Baldwin *et al.*, 2011). generally, all films are better barriers to O_2 than CO_2 which is in agreement with findings of García *et al.* (1999). It was observed that sorbitol and glycerol addition reduced gas permeability. So the rate of respiration was found to be decreased with the addition of plasticizers (Šuput *et al.*, 2013). It is observed that the addition of glycerol was found to decrease the rate of respiration considerably.

There was decrease in rate of respiration with addition of plasticizer as they improve integrity and avoid pores and cracks and thus promotes an effective barrier to gas exchange (García *et al.*, 1999). Due to acid alcohol modification of the starch, the amylose content decreases (Wang and Copeland, 2015). For the good quality of the film formation, amylose content should be high (Lourdin *et al.*, 1995). The rate of the respiration might have increased because of the improper film formation in AATS rather than other treatments.

It is found that the use glycerol has reduced the rate of respiration rather than sorbitol which might be due to higher efficiency of plasticizing by glycerol because of the smaller molar mass of the glycerol (92.0928 g/mol) in compared to sorbitol (182 g/mol) which facilitates easy interaction between glycerol starch molecular chain. (Sanyang *et al.*, 2015a).

4.4 Chlorophyll retention with starch coat in *Chaenomeles japonica*

Chlorophyll content of coated and control fruit continuously decreases during the storage time; however, the chlorophyll content was significantly affected by coating treatments. The chlorophyll content (mg/100 g) for *Chaenomeles japonica* was determined for 3 respective days by uniform coating with different forms of starches treated with glycerol and sorbitol at different concentration. The value of chlorophyll content of sample on day 1 was found to be (2.523± 0.070633 mg/100 g). This value was the reference value for the values that were observed on subsequent days for the effect of coating on retention of chlorophyll. The fruits that were studied for the chlorophyll content on subsequent days were all coated with starch coating whereas the reference sample was not coated with any starch coating.

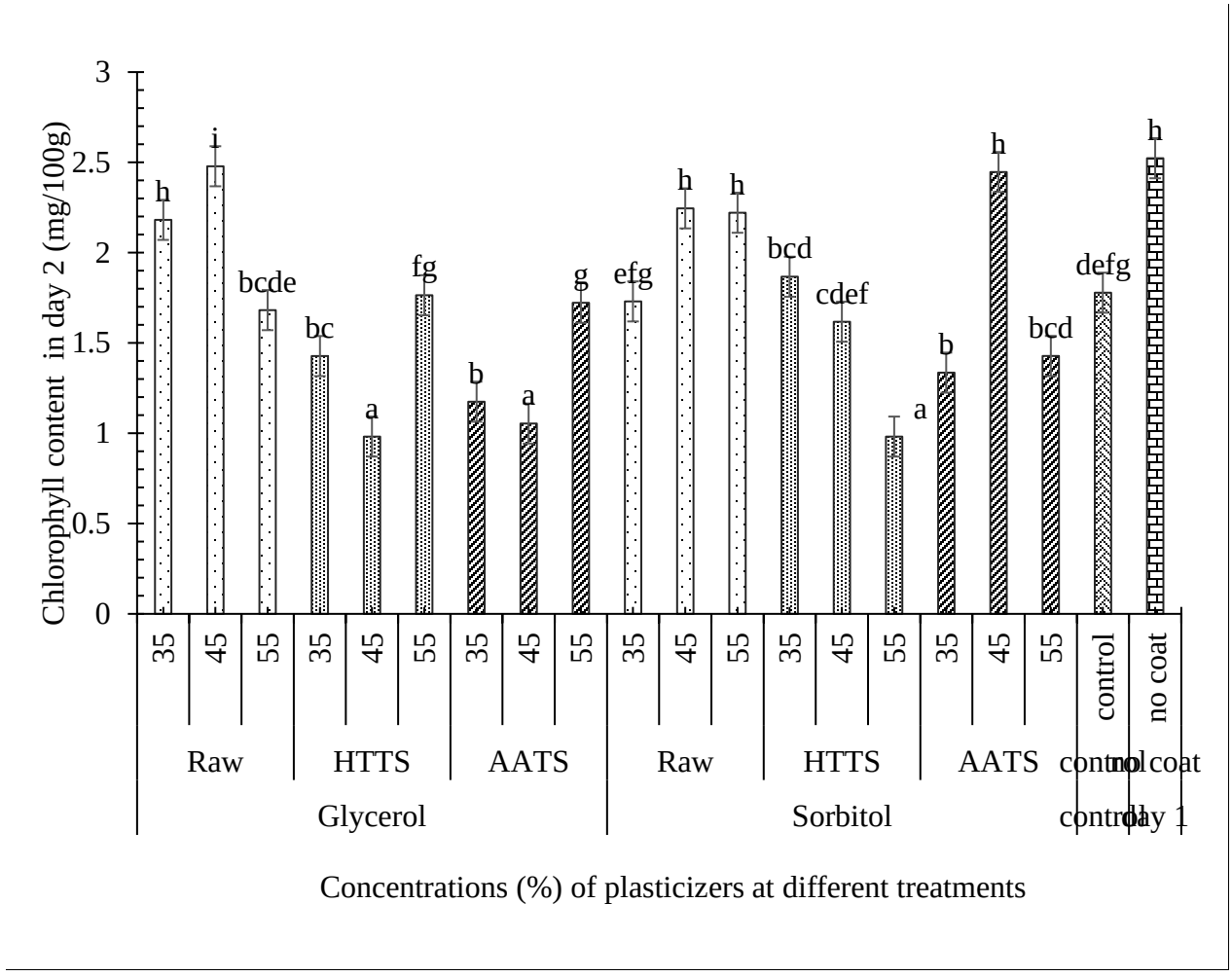


Fig. 4. 5 Chlorophyll content of coated *Chaenomeles japonica* in day 2

From Fig. 4.5, it was found that the chlorophyll has maximum value (2.4785 ±0.13902 mg/100 g) with non-treated starch plasticized with 45% glycerol and minimum value (0.98152±0.06078 mg/100 g) with HTT treated starch plasticized with 45% glycerol and HTT treated starch plasticized with 55% sorbitol. There is significant difference observed between maximum and minimum values.

It was observed from the Fig. 4.5, the sample with no coating has a value of (2.523±0.0706 mg/100 g) and control sample has a value of (1.7784±0.199 mg/100 g). These both are significantly different from one another. This is because sample with no coating represents fresh sample and the control represents the sample that has been kept until the following day.

On comparing the data of non-treated starch plasticized with 45% glycerol, with no coating and with the control sample, it was found that there are significant differences between them. However, this maximum value is significantly greater than the control and lesser than the no coat (fresh) sample. It is greater than the control sample, which suggests that the coating had significant effect in retention of chlorophyll content.

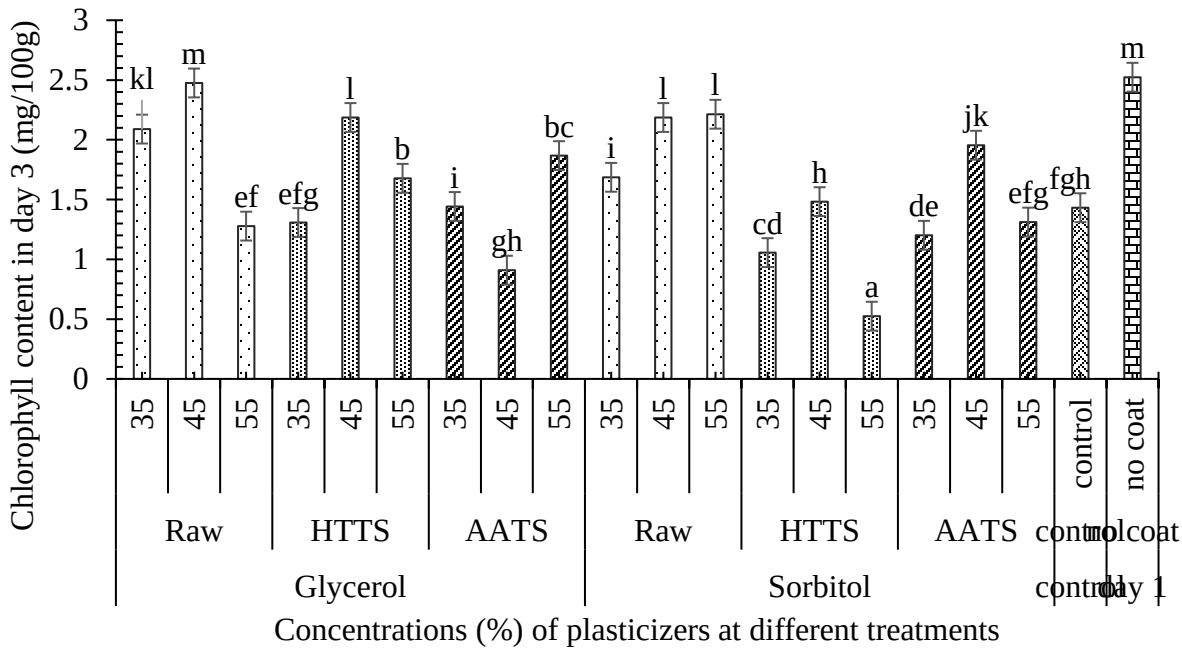


Fig. 4. 6. Chlorophyll content of coated *Chaenomeles japonica* in day 3

From Fig. 4.6, it was found that the chlorophyll has maximum value (2.47521 ±0.02859 mg/100 g) in non-treated starch plasticized with 45% glycerol and also somewhat high in HHT treated starch plasticized with 45% glycerol and non-treated starch plasticized with 55% sorbitol and minimum value (0.5246±0.046143 mg/100 g) in HTT treated starch plasticized with 55% sorbitol. There is significant difference observed between maximum and minimum values.

It was found from the Fig. 4.6 the sample with no coating has a value of (2.523±0.0706 mg/100 g) and control sample has a value of (1.43192±0.032932 mg/100 g). These both are significantly different from one another. This is because sample with no coating represents fresh sample and the control represents the samples that has been kept until the following day.

On comparing data of raw starch plasticized with 45% glycerol, with no coating and with the control sample, we can see that there are significant differences between them. However, this maximum value is significantly greater than the control and lesser than the no coat (fresh) sample. It is greater than the control sample which suggests that the coating had significant effect in retention of chlorophyll content.

It was found from table A.11, that the use of plasticizer has no significant effect in chlorophyll retention of *Chaenomeles japonica*. Only Plasticizers had no significant effect in chlorophyll retention whereas treatment only and combination of plasticizers and treatment had significant effects on chlorophyll retention. The results obtained is justified by Sanyang *et al.* (2015b). On increment of plasticizer concentration, the average amount of starch molecules binding with the plasticizer increases; and in case there has been non uniform mixing of starch, there results non uniform binding of starch and plasticizer thereby making the layer porous. So, either of plasticizers can be used; but not in a very high concentration. However, when the treatments and plasticizers interact with each other they have significant effects on chlorophyll retention. Out of these treatments, raw sample has the best results because amylose content in other treatments decrease significantly. According to Lourdin *et al.* (1995) to form a good film, good amount of amylose is required. It was also seen that, among the plasticizers used for experiment, glycerol has the tendency to form best film. This could be because of its lower molar mass compared to sorbitol (Sanyang *et al.*, 2015a).

Moving on to the total interaction of all the factors, it was found that non-treated starch plasticized with 45% glycerol has most significant positive effect on retention of chlorophyll. Sanyang *et al.* (2015a) mentions about the possibility of non-uniform mixing of the plasticizer to obtain the results as they obtained it. Our mixing could have been more uniform compared to Sanyang *et al.* (2015a). So, we obtained best results in 45% glycerol rather than 35% glycerol.

It can be concluded with evidence form above data that the presence of coating significantly helps in chlorophyll retention. This could be because the presence of coating hinders respiration rate thereby preventing the substitution of Mg in chlorophyll molecule by Hydrogen. This helps in prevention of change of chlorophyll into other pigments (Baldwin *et al.*, 1994).

4.5 Vitamin A retention with starch coat in *Chaenomeles japonica*

Vitamin A content of coated and control fruit continuously decreases during the storage time; however, the vitamin A content was significantly affected by coating treatments. The vitamin A content (μg/100 g) for *Chaenomeles japonica* was determined for 3 respective days by uniform coating with different forms of starches treated with glycerol and sorbitol at different concentration. The value of Vitamin A content of *Chaenomeles japonica* on day 1 is (0.498898 ± 0.010577 μg/100 g). This value was considered as the reference value for the values that were observed on subsequent days for the effect of coating on retention of Vitamin A. The fruits that were studied for the Vitamin A content on subsequent days were all coated with starch coating whereas the reference sample was not coated with any starch coating.

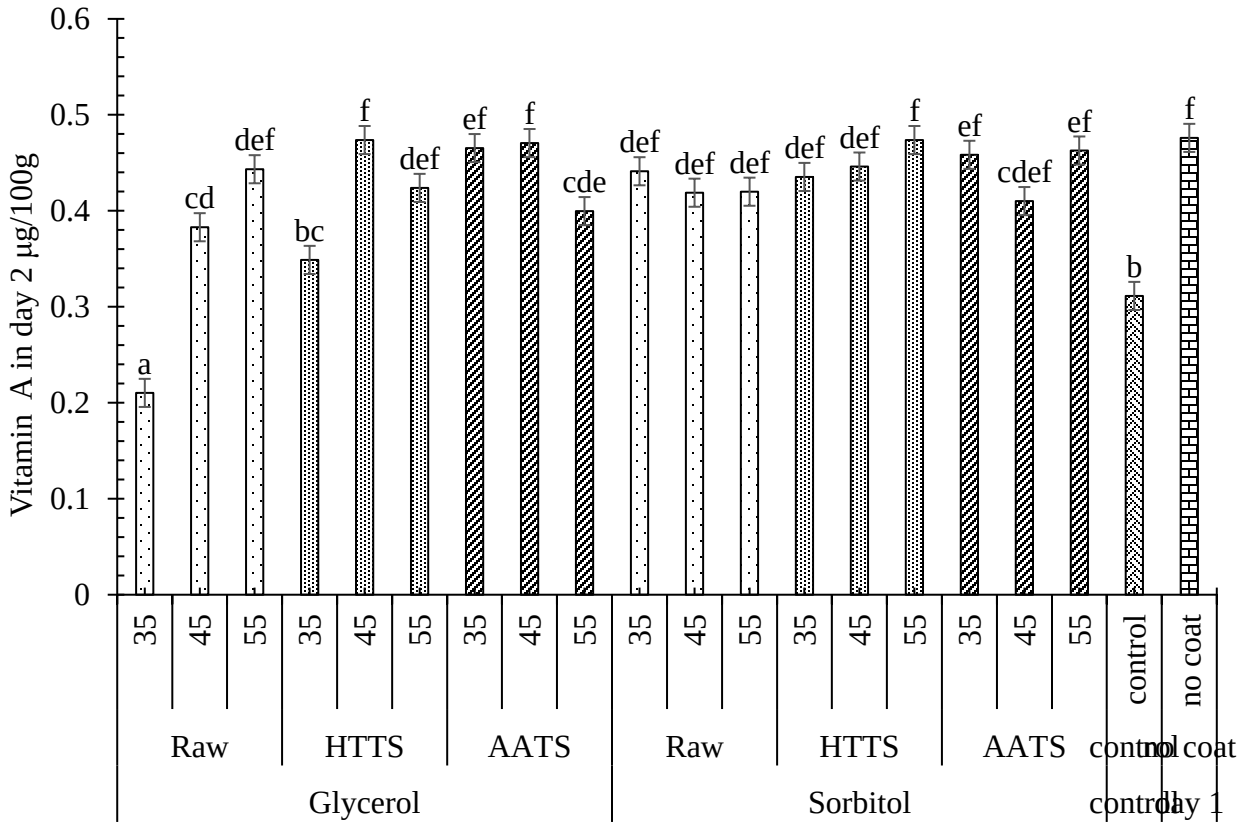


Fig. 4.7. Vitamin A content of coated *Chaenomeles japonica* in day 2

It was found in Fig. 4.7, there are significant effects of all sources of variation at every level of interactions because all F. Pr values are < 0.05. IF we look at LSD test, treatment with HTT sorbitol 55% shows maximum retention of the vitamin. However, it is significant compared to HTT glycerol 45%, AAT glycerol 45%, AAT glycerol 35%, AAT sorbitol 55% and AAT sorbitol 45%, sorbitol 35%, HTT sorbitol 55%, raw sorbitol 55, HTT sorbitol 45%, raw glycerol 55%. Similarly, AAT glycerol 55%, AAT sorbitol 45%, raw sorbitol 45%, raw sorbitol 55%, HTT glycerol 55%, HTT sorbitol 35%. raw sorbitol 35%, HTT sorbitol 45%, AAT sorbitol 55%, AAT glycerol 35% are also not significantly different from each other. Also, treatments raw glycerol 45%, AAT glycerol 55%, AAT sorbitol 45%, raw sorbitol 45%, raw sorbitol 55%, HTT glycerol 55%, HTT sorbitol 35%. raw sorbitol 35%, HTT sorbitol 45% have no significant effects. Similarly, treatments HTT glycerol 35%, raw glycerol 45%, AAT glycerol 55%, AAT sorbitol 45% are also not significantly different from each other. We can also see that control and HTT glycerol 35% have no significant differences between other. However, all these values are significantly different from raw glycerol 35%. raw glycerol 35% has the most % loss. On comparing the control sample with HTT sorbitol 55%, there is a high significant difference.

From Fig. 4.7, it was found that the vitamin A has maximum value (0.473734 ± 0.002639 μg/100 g) in HTT treated starch plasticized 45% glycerol and HTT treated starch plasticized with 55% sorbitol and minimum value (0.21029 ± 0.091959 μg/100 g) in non-treated starch plasticized with 35% glycerol. There is significant difference observed between maximum values and the minimum value.

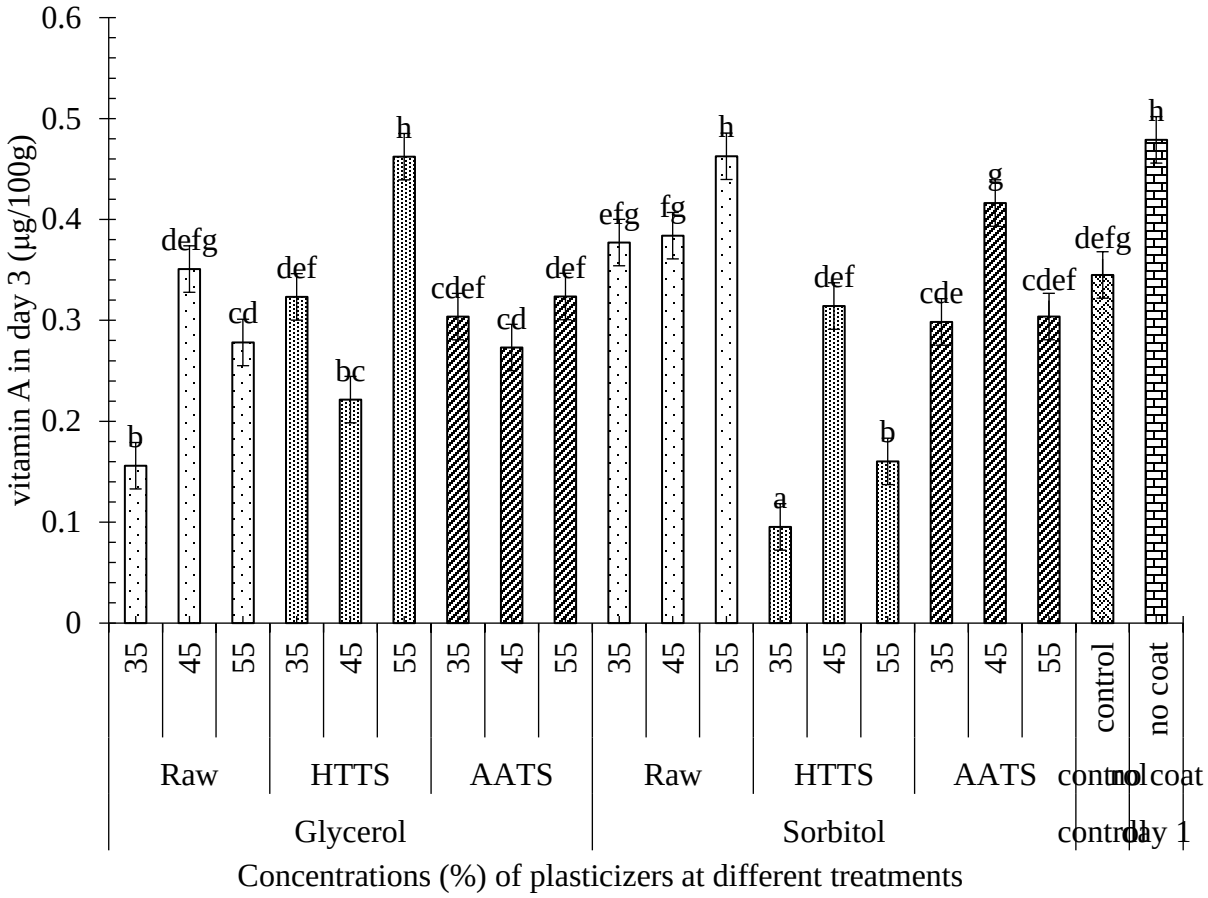


Fig. 4.8. Vitamin A content of coated *Chaenomeles japonica* in day 3

It was found in Fig. 4.8, out of different sources of variations, only plasticizers have no significant effect on vitamin retention whereas all other levels of treatments have significant effects on vitamin retention. The samples that are not significantly different from each other are raw glycerol 35%, HTT sorbitol 55%, HTT glycerol 45%. Similarly, AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 35%, AAT sorbitol 55% are also not significantly different from each other. Also, AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 55%, HTT sorbitol 45%, HTT glycerol 35%, control and raw glycerol 45% are indifferent. Similarly, AAT sorbitol 35%, AAT glycerol 35%, AAT sorbitol 55% are also indifferent. The other treatments that are insignificant are AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 55%, HTT sorbitol 45%, HTT glycerol 35%, control and raw glycerol 45%, raw sorbitol 35%. AAT glycerol 35%, AAT sorbitol 55% are

also not significantly different from each other. Similarly, AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 55%, HTT sorbitol 45%, HTT glycerol 35%, control and raw glycerol 45%, raw sorbitol 35%, raw sorbitol 45% are also indifferent. Lastly, control and raw glycerol 45%, raw sorbitol 35%, raw sorbitol 45%, AAT sorbitol 45% are also not significantly different from each other.

It was observed in Fig. 4.8 that the HTT treated starch plasticized with 55% glycerol (0.482193±0.05932 μg/100 g) has most significant effect on vitamin retention. However, the HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol are not different form each other. These treatments are however significantly different from the control sample.

The retention of nutritional factor such as vitamin, retinol depends upon the respiration rate of fruits and vegetable (Greenwood, 2019). From the above result of respiration and vitamin, we can conclude that lower respiration rate was determined in HTTS glycerol 35%. HTTS glycerol 45% and HTTS glycerol 55% have a little bit high respiration as compared to HTTS 35% but lower than other treatment. The retention of vitamin can be explained by decrement in the respiration rate. Lower the value of respiration rate higher was the vitamin retention.

Coating on fruits represents the packaging material which act as the barrier for permeability of gases such O_2 and CO_2 and act as a modified storage (MAP). Low concentration of O_2 enhances the retention of vitamin because higher O_2 concentration leads to the higher respiration and higher production of heat and it may cause the depletion of nutritional parameters of fruits (Baldwin *et al.*, 1994).

Vitamin is directly related with light, O_2, moisture content and pH of produce. By the coating on produce, the coating serves as barrier to the moisture and light. Coating prevents direct contact with light by which vitamin depletion can be minimized. Coating can also act as a barrier to the moisture which can reduce the vitamin depletion (Barrett, 2018).

It has been extensively studied that fat-soluble Vitamin content of various vegetables are heat dependent. It has been explained by Lešková *et al.* (2006) that vegetables and fruits processed by applying heat treatments had reduced vitamin content than with commodities with no heat

treatment. So, it can be inferred without a doubt that heat of respiration also results in similar phenomenon. This means that decrement of heat of respiration due to reduction in respiration rate (by biofilm coatings) ultimately increases vitamin retention.

It was observed that sorbitol and glycerol addition reduced gas permeability. So the rate of respiration was found to decrease with the addition of plasticizer (García *et al.*, 1999).

The water binding capacity of samples treated with HTT increased which might be because hydrothermal treatment (HTT) increases the hydrophilic tendency of starches. Similar results were explained by Abraham (1993).

4.6 Retinol retention with starch coat in *Chaenomeles japonica*

Retinol content of coated and control fruit continuously decreases during the storage time; however, the retinol content was significantly affected by coating treatments. The retinol content (μg/100 g) for *Chaenomeles japonica* was determined for 3 respective days by uniform coating with different forms of starches treated with glycerol and sorbitol at different concentration. The value of retinol content of *Chaenomeles japonica* on day 1 is (1.58912± 0.035256 μg/100 g). This value was taken as the reference value for the values that were observed on subsequent days for the effect of coating on retention of retinol. The fruits that were studied for the Retinol content on subsequent days were all coated with starch coating whereas the reference sample was not coated with any starch coating.

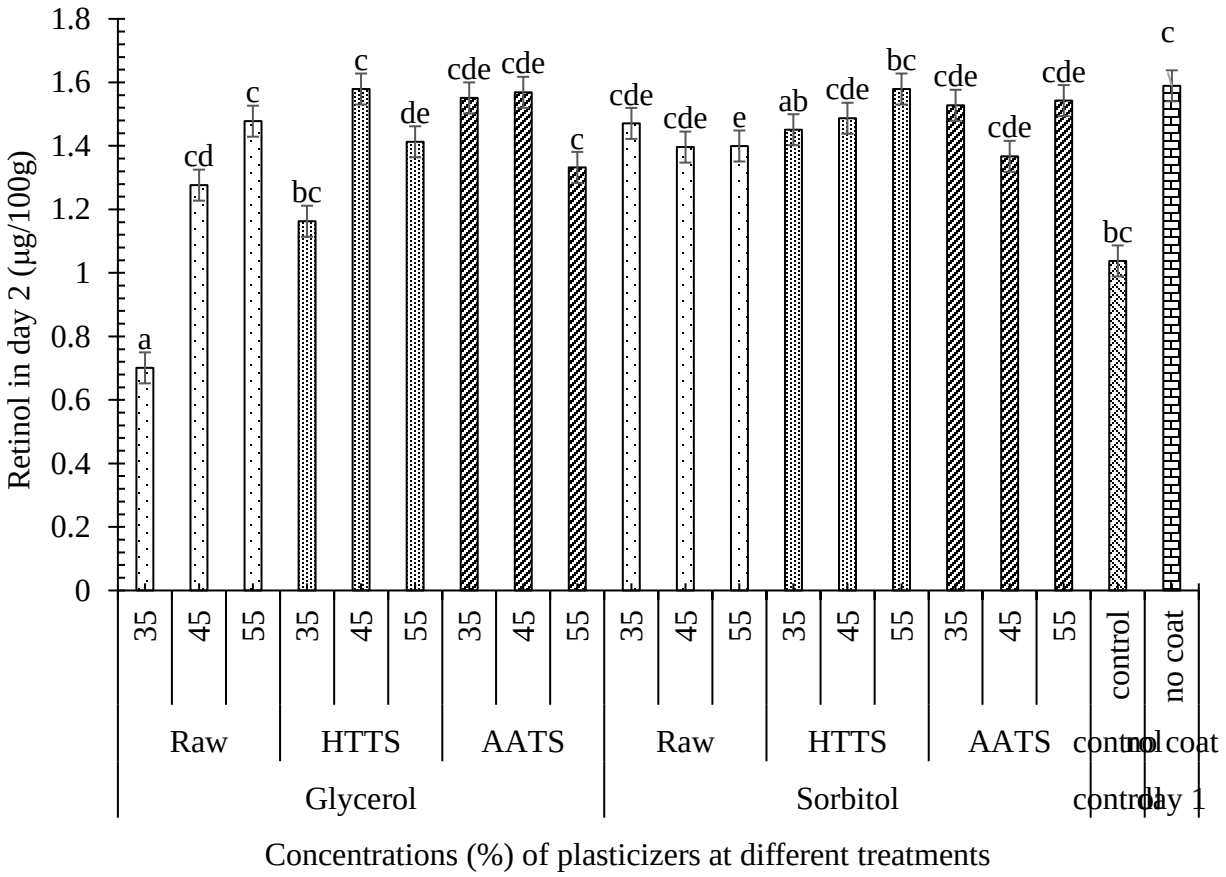


Fig. 4.9. Retinol content of coated *Chaenomeles japonica* in day 2

It was observed in Fig. 4.9 that source of variation that had significant effect on retinol change were interactions between treatment and plasticizers and only concentration.

It was observed in Fig. 4.9 that most significant effect was seen in treatment raw sorbitol 55%. However, this treatment even though was significantly different from control sample, was not significantly different from some other treatment methods AAT glycerol 45%, AAT sorbitol 35%, HTT sorbitol 45%, AAT sorbitol 55%, AAT glycerol 35%, raw sorbitol 45%, raw sorbitol 35%, AAT sorbitol 45%, HTT glycerol 55%. Similarly, raw glycerol 45%, AAT glycerol 45%, AAT sorbitol 35%, HTT sorbitol 45%, AAT sorbitol 55%, AAT glycerol 35%, raw sorbitol 45%, raw sorbitol 35%, AAT sorbitol 45%, HTT glycerol 55% are also not significantly different from each other. Similarly, HTT sorbitol 55%, control, HTT glycerol 35%, HTT glycerol 45%, raw glycerol 55%, AAT glycerol 55%, raw glycerol 45%, AAT glycerol 45%, AAT sorbitol 35%, HTT sorbitol 45%, AAT sorbitol 5%5, AAT glycerol 35%, raw sorbitol 45%, raw sorbitol 35%, AAT sorbitol 45% are also not significantly different from each other. The other treatments not different from each other are HTT sorbitol 35%, HTT sorbitol 55%, control, HTT glycerol 35%. The last group which are insignificant from each other are raw glycerol 35% and HTT sorbitol 55%.

From Fig. 4.9, it was observed that the retinol has maximum value (1.579114±0.008795 μg/100 g) in HTT treated starch plasticized with 45% glycerol and minimum value (0.700967±0.306529 μg/100 g) in non-treated starch plasticized with 35% glycerol. There is significant difference observed between these treatments.

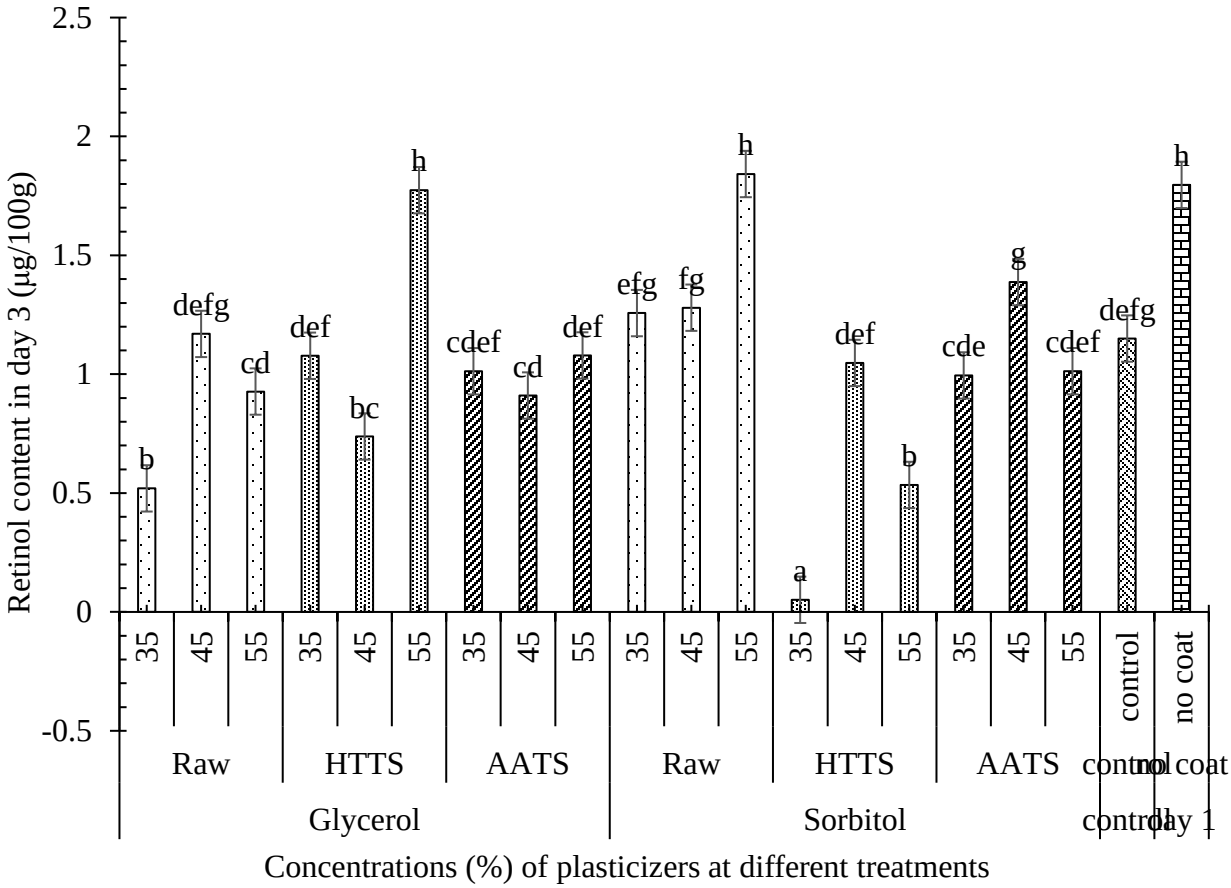


Fig. 4.10 Retinol content of coated *Chaenomeles japonica* in day 3

It was observed in Fig. 4.10, that most of the sources of variation that had significant effect on retinol change except plasticizers alone.

It was observed in Fig. 4.10, that the treatments that are insignificant from each other are raw glycerol 35%, HTT sorbitol 55%, HTT glycerol 45%. Similarly, HTT glycerol 45%, AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 35%, AAT sorbitol 55% are insignificant from each other. AAT glycerol 45%, raw glycerol 55%, AAT sorbitol 35%, AAT glycerol 35%, AAT sorbitol 55%, HTT sorbitol 45%, HTT glycerol 35%, AAT glycerol 55%, control, raw glycerol 45%. Similarly, AAT sorbitol 35%, AAT glycerol 35%, AAT sorbitol 55% are insignificant from each other. Similarly, AAT glycerol 35%, AAT sorbitol 55%, HTT sorbitol 45%, HTT glycerol 35%, AAT glycerol 55%, control, raw glycerol 45%, raw sorbitol 35%, raw sorbitol 45% are insignificant to each other. Similarly control, raw glycerol 45%, raw

sorbitol 35%, raw sorbitol 45%, AAT sorbitol 45% are insignificant to each other. Lastly, treatments HTT glycerol 55%, raw sorbitol 55% are also insignificant from each other.

The treatments that are significantly different from each other are: glycerol raw 35%, glycerol raw 55%, glycerol HTTS 55%, raw sorbitol 35%, HTTS sorbitol 35%.

In Fig. 4.10, it was observed that the retinol has maximum value (1.773977475±0.197733 μg/100 g) in HTT treated starch plasticized with 55% glycerol and minimum value (0.051044272± 0.00574 μg/100 g) in HTT treated starch plasticized with 35% sorbitol There is significant difference observed between these treatments.

Retinol was calculated by dividing vitamin A content by 0.3 as mentioned in material and method. retinol is preformed vitamin A that can be directly absorbed in the body (Ball, 2006). Therefore, changes in retinol and Vitamin A are proportional to each other. The values obtained in the graph above Fig. 4.10, confirm this fact. The treatment that had similar effects on change of both Vitamin A and retinol are HTT glycerol 55%, raw sorbitol 55%.

Therefore, reasons that hold true for Vitamin A also hold true for retinol change.

4.7 β-Carotene retention with starch coat in *Chaenomeles japonica*

β-carotene content of coated and control fruit continuously decreases during the storage time; however, the β-carotene content was significantly affected by coating treatments. The β-carotene content (μg/100 g) for *Chaenomeles japonica* was determined for 3 respective days by uniform coating with different forms of starches treated with glycerol and sorbitol at different concentration. The value of β-carotene content of *Chaenomeles japonica* on day 1 is (0.79981 ± 0.017628 μg/100 g). This value was taken as the reference value for the values that were observed on subsequent days for the effect of coating on retention of β-carotene. The fruits that were studied for the β-carotene content on subsequent days were all coated with starch coating whereas the reference sample was not coated with any starch coating.

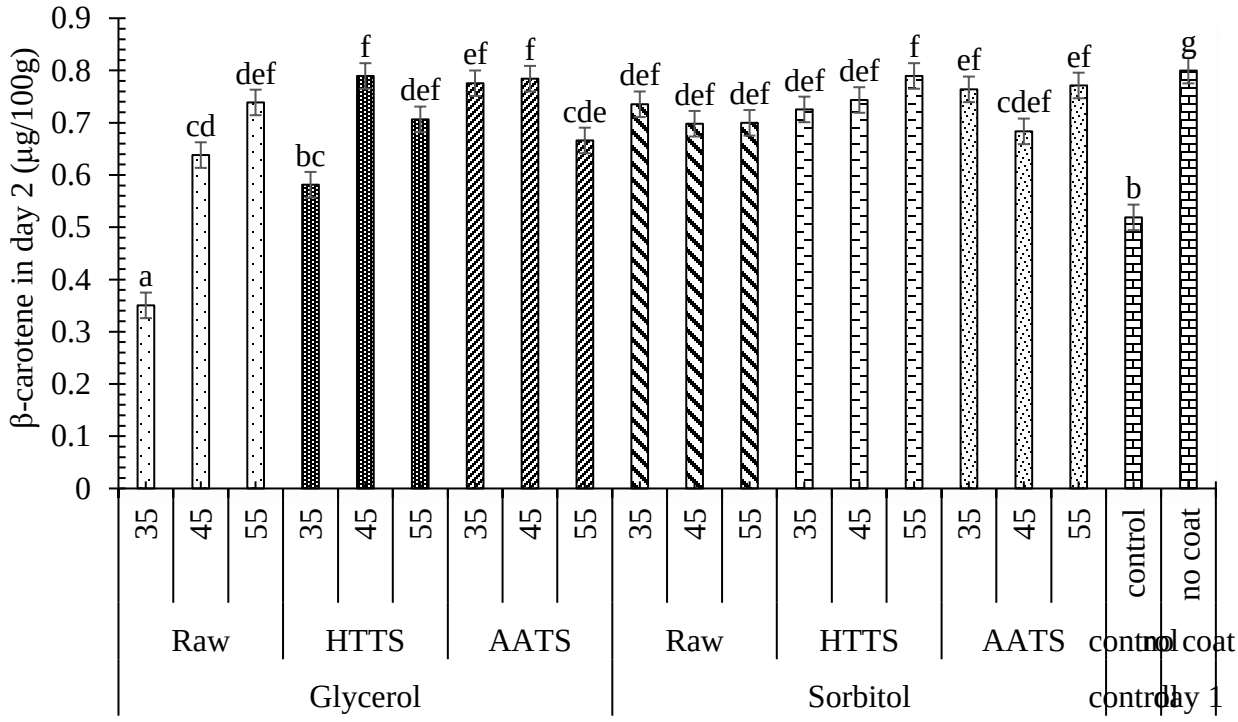
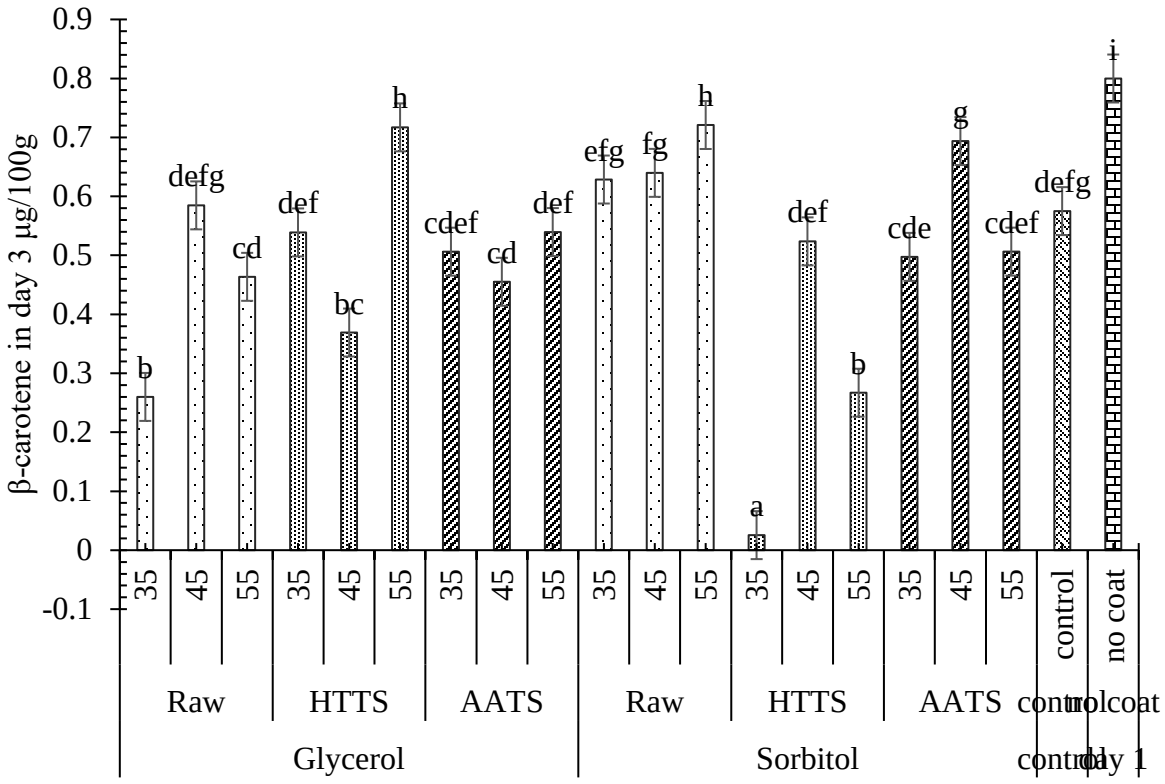


Fig. 4. 11 β-carotene content of coated *Chaenomeles japonica* in day 2

In Fig. 4.11, it was observed that the carotene has maximum value (0.789557±0.004398 μg/100 g) in HTT treated starch plasticized with 45% glycerol

, AATS treated 45% glycerol and HTTS treated 55% sorbitol and minimum value (0.350483±0.153265 μg/100 g) in non-treated starch plasticized with 55% glycerol There is significant difference observed between these treatments.

In the Fig., the bars with same alphabets are insignificant from each other. So, the treatments that are completely different from each other are raw glycerol 35%, HTTS glycerol 35%, HTTS glycerol 45% and 55%, AATS glycerol 35% control sample and no coat sample. The highest retention is observed in HTTS treatment plasticized with 45% glycerol, AATS treated 45% glycerol and HTTS treated 55% sorbitol. And minimum has been seen in raw starch plasticized with 55% glycerol. These maximum and minimum values are significantly different from the control and no-coat samples.



Concentrations (%) of plasticizers at different treatments

Fig. 4.12 β-carotene content of coated *Chaenomeles japonica* in day 3

It was observed from Fig. 4.12 that the bars with same alphabets are insignificant from each other. So, the treatments that are completely different from each other are raw glycerol 35%, raw glycerol 55%, HTTS glycerol 45% and 55%, AATS glycerol 35%, HTTS sorbitol 35%, AATS Sorbitol 45%, control sample and no coat sample. The highest retention of β-carotene is (0.721014± 0.206253 µg/100 g) observed in HTTS treatment plasticized with 55% glycerol and raw treated 55% sorbitol And minimum value (0.025522±0.00287 µg/100 g) has been seen in HTT starch plasticized with 35% sorbitol These maximum and minimum values are significantly different from the control and no-coat samples.

One molecule of β carotene gives two molecules of vitamin A (Ophardt and Emeritus, 2019). Therefore, change in these compounds are directly proportional to each other. On the other hand,

retinol is preformed vitamin A that can be directly absorbed in the body. Therefore, changes in retinol and Vitamin A are also proportional to each other.

Therefore, reasons that hold true for Vitamin A also hold true for β- carotene change.

Part-V

Conclusion and recommendations

5.1 Conclusions

On the basis of the work performed, following conclusions were made.

1. Modifications improved the physicochemical properties of starch.
2. Acid alcohol treatment improved the oil absorbing capacity, solubility and iodine affinity of starch.
3. Hydrothermal treatment improved the water binding capacity (WBC), dispersibility.
4. The rate of respiration was decreased with the starch coat in *Chaenomeles japonica*. In day 1 HTT treated starch plasticized with 35% glycerol had lowest rate of respiration than non-coated fruit. Non-coated fruit had the maximum 2216.667 rate of respiration in day 3 compared to all coated fruits.
5. The chlorophyll had maximum value in non-treated starch plasticized with 45% glycerol (2.47521±0.02859 mg/100 g) and minimum value in HTT treated starch plasticized with 55% sorbitol (0.5246±0.046143 mg/100 g).
6. The HTT treated starch plasticized with 55% glycerol (0.532193±0.05932 μg/100 g) had most significant effect on vitamin retention. However, HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol were not different form each other. These treatments were however significantly different from the control sample.
7. The treatment that had similar effects on change of both Vitamin A and retinol were HTT glycerol 55%, raw sorbitol 55%.
8. The highest retention of β carotene was observed in HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol (0.721014±0.206253 μg/100 g). And minimum value was seen in HTT treated starch plasticized with 35% sorbitol

(0.025522±0.00287 μg/100 g). These maximum and minimum values were significantly different from the control and no-coat samples.

9. From the overall view of data analysis, HTT treated starch plasticized with glycerol coating had maximum effect on nutrients retention. Concentrations might be varied.

5.2 Recommendations

1. Biodegradable films can be prepared from other starch rich sources with use of different plasticizers.
2. Biodegradable films can be used as the coating materials for different fruits and vegetables
3. Practical application of biodegradable films can be made on different food and non-food materials.

Part-VI

Summary

In the study, two techniques viz. hydrothermal treatment (moisture adjusted to 28% and heated at 110°C for 3 h.) and acid-alcohol treatment (treated with 100 ml of rectified alcohol and 20 ml of conc. HCl) were applied for the modification of the extracted potato starch and their functional properties were studied. Modified as well as extracted starches with varied glycerol concentration (35%, 45%, 55%) were used for the preparation for starch-based suspensions to be used as films after dipping *Chaenomeles japonica* (Maule's quince) in it.

Different modifications ($p<0.05$) had significant effects. Acid alcohol treatment significantly improved the oil absorbing capacity (136±3.6%), solubility (13.83±0.33%), and iodine affinity (320±5 ppm) of starch. Hydrothermal treatment improved the water binding capacity (277±5.04%) (WBC), dispersibility (69±3.6%), wettability (11±1 min) of potato starch. Non-coated fruit had the maximum rate of respiration—2216.667 mg CO_2/ kg/hr in day 3 compared to all coated fruits. The rate of respiration significantly ($p<0.05$) decreased with the starch coat in *Chaenomeles japonica*. The chlorophyll had maximum value (2.47521±0.02859 mg/100 g) in non-treated starch plasticized with 45% glycerol and minimum value (0.5246±0.046143mg/100g) HTT treated starch plasticized with 55% sorbitol. The vitamin retention was maximum (0.532193±0.05932 μg/100 g) with HTT treated starch plasticized with 55% glycerol. However, HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol were not different form each other. These treatments were however significantly different from the control sample. The treatment that had similar effects on change of both Vitamin A and retinol are HTT treated starch plasticized with 55% glycerol and non-treated starch plasticized with 55% sorbitol. The highest retention of carotene (0.721014± 0.206253 μg/100 g) is observed in HTT treated starch plasticized with 55% glycerol and non-treated starch treated with 55% sorbitol and minimum retention (0.025522±0.00287 μg/100 g) has been found in HTT treated starch plasticized with 35% sorbitol. These maximum and minimum values were significantly different from the control and non-coated samples of day one.

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Appendices

Appendix A

Variate: Bulk Density

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	0.016350	0.008175	1.41	0.315
Residual	6	0.034800	0.005800		
Total	8	0.051150			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
raw	0.7300	a
aa	0.7400	a
htt	0.8250	a

Variate: Dispersibility

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	486.000	243.000	34.71	<.001
Residual	6	42.000	7.000		
Total	8	528.000			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
raw	51.00	a
aa	60.00	b
htt	69.00	c

Variate: Iodine affinity of starch

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	62784.22	31392.11	1116.72	<.001
Residual	6	168.67	28.11		
Total	8	62952.89			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
aa	320.0	a
htt	496.0	b
raw	498.3	b

Variate: Oil_absorption_capacity

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	13392.89	6696.44	407.22	<.001
Residual	6	98.67	16.44		
Total	8	13491.56			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
aa	136.0	a
htt	174.7	b
raw	230.0	c

Variate: Water binding capacity

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	11116.52	5558.26	190.50	<.001
Residual	6	175.07	29.18		
Total	8	11291.58			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
aa	191.1	a
raw	239.4	b
htt	277.0	c

Variate: paste_clarity

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	2	249.609	124.804	86.14	<.001
Residual	6	8.693	1.449		
Total	8	258.302			

Tukey's 95% confidence intervals

Treatments	Mean	S. D. Indicator
aa	18.00	a
htt	19.07	a
raw	29.67	b

Analysis of variance

Variate: Respiration Rate in day 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	342246.	114082.	61.77	<.001
Plasticizers	2	423674.	211837.	114.69	<.001
conccentration	3	12989.	4330.	2.34	0.088
Treatments.Plasticizers	1	37816.	37816.	20.47	<.001
Treatments.conccentration	3	4078.	1359.	0.74	0.537
Plasticizers.conccentration	1	22856.	22856.	12.37	0.001
Treatments.Plasticizers.conccentration	5	15704.	3141.	1.70	0.158
Residual	38	70187.	1847.		
Total	56	929550.			

Fisher's unprotected least significant difference test

Variate: Respiration Rate in day 1

Treatments: Plasticizers.conccentration

Treatments	Mean	S. D. Indicator
HTTS glycerol 0.35	51.1	a
HTTS glycerol 0.55	61.8	ab
Raw glycerol 0.35	72.1	ab
HTTS glycerol 0.45	74.6	ab
Raw glycerol 0.55	99.5	abc
Raw glycerol 0.45	127.3	bc
AATS glycerol 0.55	158.1	cd
Raw sorbitol 0.35	211.2	de
AATS glycerol 0.35	241.0	ef
AATS glycerol 0.45	253.3	efg
Raw sorbitol 0.45	277.4	efgh
HTTS sorbitol 0.35	280.8	efgh
Raw sorbitol 0.55	281.8	efgh
AATS sorbitol 0.35	303.7	fgh
AATS sorbitol 0.45	314.1	gh
HTTS sorbitol 0.45	321.4	gh
HTTS sorbitol 0.55	334.3	h
AATS sorbitol 0.55	408.7	i
control No 0.00	498.7	j

Analysis of variance

Variate: Respiration Rate in day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	286098.	95366.	31.55	<.001
Plasticizers	2	171632.	85816.	28.39	<.001
conccentration	3	37412.	12471.	4.13	0.013
Treatments.Plasticizers	1	105502.	105502.	34.90	<.001
Treatments.conccentration	3	323540.	107847.	35.68	<.001
Plasticizers.conccentration	1	118595.	118595.	39.23	<.001
Treatments.Plasticizers.conccentration	5	53166.	10633.	3.52	0.010
Residual	38	114868.	3023.		
Total	56				

Fisher's unprotected least significant difference test

Variate: Respiration Rate in day 2

Treatments: Plasticizers.conccentration

Treatments	Mean	S. D. Indicator
AATS glycerol 0.45	634.5	a
HTTS glycerol 0.35	673.0	ab
Raw glycerol 0.35	710.4	abc
Raw sorbitol 0.35	728.2	bcd
HTTS glycerol 0.45	759.1	bcde
Raw sorbitol 0.55	785.5	cdef
Raw glycerol 0.45	797.4	cdefg
AATS sorbitol 0.45	818.7	defg
AATS sorbitol 0.55	835.3	efgh
AATS glycerol 0.55	838.5	efgh
HTTS glycerol 0.55	867.0	fgh
Raw glycerol 0.55	883.9	ghi
HTTS sorbitol 0.55	910.1	hij
Raw sorbitol 0.45	914.0	hij
HTTS sorbitol 0.45	972.4	ij
AATS glycerol 0.35	977.6	j
AATS sorbitol 0.35	1093.7	k
HTTS sorbitol 0.35	1098.3	k
control No 0.00	1131.7	k

Analysis of variance

Variate: Respiration Rate in day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	3497177.	1165726.	341.82	<.001
plasticizers	2	107750.	53875.	15.80	<.001
Concentration	3	206196.	68732.	20.15	<.001
Treatment.plasticizers	1	503183.	503183.	147.54	<.001
Treatment.Concentration	3	1523135.	507712.	148.87	<.001
plasticizers.Concentration	1	45446.	45446.	13.33	<.001
Treatment.plasticizers.Concentration	5	660899.	132180.	38.76	<.001
Residual	38	129594.	3410.		
Total	56	6673380			

.

Fisher's unprotected least significant difference test

Variate: Respiration Rate in day 3

Treatment: plasticizers.Concentration

Treatments	Mean	S. D. Indicator
HTTS glycerol 0.35	806	a
Raw sorbitol 0.35	834	ab
HTTS glycerol 0.55	930	bc
AATS glycerol 0.45	1002	cd
Raw sorbitol 0.45	1030	de
Raw glycerol 0.45	1051	def
Raw glycerol 0.35	1088	defg
AATS sorbitol 0.55	1093	defg
AATS glycerol 0.55	1106	efg
HTTS sorbitol 0.55	1136	fgh
HTTS glycerol 0.45	1151	gh
Raw glycerol 0.55	1156	gh
AATS sorbitol 0.45	1214	h
HTTS sorbitol 0.45	1338	i
Raw sorbitol 0.55	1366	i
AATS sorbitol 0.35	1468	j
HTTS sorbitol 0.35	1496	j
AATS glycerol 0.35	1881	k
control No 0.00	2217	l

Analysis of variance

Variate: Total_cholorophyll retention in day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	10.73946	3.57982		<.001
plasticizers	2	0.10552	0.05276	1.24	0.294
concn	3	0.73522	0.24507	5.76	0.001
treatment.plasticizers	1	0.52271	0.52271		<.001
treatment.concn	3	1.96889	0.65630		<.001
plasticizers.concn	1	2.99784	2.99784		<.001
treatment.plasticizers.concn	5	7.80484	1.56097		<.001
Residual	95	4.03922	0.04252		
Total	113	28.91369			

Fisher's unprotected least significant difference test

Variate: Total_cholorophyll retention in day 2

Treatment: plasticizers.concn

Treatments	Mean	S. D. Indicator
HTT sorbitol 55	0.753	a
HTT glycerol 45	0.916	a
AAT glycerol 45	0.982	a
AAT sorbitol 35	1.269	b
AAT glycerol 35	1.308	b
HTT glycerol 35	1.368	bc
AAT sorbitol 55	1.370	bcd
HTT sorbitol 35	1.462	bcd
raw glycerol 55	1.480	bcde
HTT sorbitol 45	1.550	cdef
control no 0	1.605	defg
raw sorbitol 35	1.708	efg
HTT glycerol 55	1.721	fg
AAT glycerol 55	1.795	g
raw glycerol 35	2.136	h
AAT sorbitol 45	2.200	h
raw sorbitol 45	2.216	h
raw sorbitol 55	2.217	h
raw glycerol 45	2.477	i

Analysis of variance

Variate: Total_cholorophyll retention in day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	6.526435	2.175478	245.24	<.001
plasticizers	2	0.013151	0.006575	0.74	0.483
concn	3	0.355575	0.118525	13.36	<.001
treatment.plasticizers	1	0.345845	0.345845	38.99	<.001
treatment.concn	3	1.001762	0.333921	37.64	<.001
plasticizers.concn	1	1.650601	1.650601	186.07	<.001
treatment.plasticizers.concn	5	4.549539	0.909908	102.57	<.001
Residual	38	0.337088	0.008871		
Total	56	14.779996			

Fisher's unprotected least significant difference test

Variate: Total_cholorophyll retention in day 3

Treatment: plasticizers.concn

Treatments	Mean	S. D. Indicator
HTT sorbitol 55	0.525	a
HTT glycerol 45	0.850	b
AAT glycerol 45	0.909	bc
HTT sorbitol 35	1.056	cd
AAT sorbitol 35	1.201	de
raw glycerol 55	1.278	ef
HTT glycerol 35	1.308	efg
AAT sorbitol 55	1.312	efg
control no 0	1.432	fgh
AAT glycerol 35	1.442	gh
HTT sorbitol 45	1.482	h
HTT glycerol 55	1.678	i
raw sorbitol 35	1.686	i
AAT glycerol 55	1.868	j
AAT sorbitol 45	1.954	jk
raw glycerol 35	2.089	kl
raw sorbitol 45	2.186	l
raw sorbitol 55	2.214	l
raw glycerol 45	2.475	m

Analysis of variance

Variate: vitamin A retention in day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	0.069159	0.023053	14.42	<.001
plasticizers	2	0.020170	0.010085	6.31	0.004
concn	3	0.021529	0.007176	4.49	0.009
treatment.plasticizers	1	0.015391	0.015391	9.63	0.004
treatment.concn	3	0.032920	0.010973	6.86	<.001
plasticizers.concn	1	0.033375	0.033375	20.87	<.001
treatment.plasticizers.concn	5	0.041446	0.008289	5.18	0.001
Residual	38	0.060757	0.001599		
Total	56	0.294748			

Fisher's unprotected least significant difference test

Variate: vitamin A retention in day 2

Treatment: plasticizers.concn

Treatments	Mean	S. D. Indicator
raw glycerol 35	0.2103	a
control no 0	0.3113	b
HTT glycerol 35	0.3488	bc
raw glycerol 45	0.3829	cd
AAT glycerol 55	0.3997	cde
AAT sorbitol 45	0.4101	cdef
raw sorbitol 45	0.4189	def
raw sorbitol 55	0.4199	def
HTT glycerol 55	0.4239	def
HTT sorbitol 35	0.4353	def
raw sorbitol 35	0.4412	def
raw glycerol 55	0.4433	def
HTT sorbitol 45	0.4461	def
AAT sorbitol 35	0.4584	ef
AAT sorbitol 55	0.4628	ef
AAT glycerol 35	0.4653	ef
AAT glycerol 45	0.4706	f
HTT glycerol 45	0.4737	f
HTT sorbitol 55	0.4737	f

Analysis of variance

Variate: vitamin A retention in day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	0.076634	0.025545	9.90	<.001
plasticizers	2	0.000591	0.000296	0.11	0.892
concn	3	0.121764	0.040588	15.73	<.001
treatment.plasticizers	1	0.318535	0.318535	123.42	<.001
treatment.concn	3	0.047825	0.015942	6.18	0.002
plasticizers.concn	1	0.046709	0.046709	18.10	<.001
treatment.plasticizers.concn	5	0.216284	0.043257	16.76	<.001
Residual	38	0.098076	0.002581		
Total	56	0.926419			

Fisher's unprotected least significant difference test

Variate: vitamin A retention in day 3

Treatment: plasticizers.concn

Treaments	Mean	S. D. Indicator
HTT sorbitol 35	0.0153	a
raw glycerol 35	0.1559	b
HTT sorbitol 55	0.1602	b
HTT glycerol 45	0.2214	bc
AAT glycerol 45	0.2731	cd
raw glycerol 55	0.2781	cd
AAT sorbitol 35	0.2984	cde
AAT glycerol 35	0.3037	cdef
AAT sorbitol 55	0.3038	cdef
HTT sorbitol 45	0.3142	def
HTT glycerol 35	0.3233	def
AAT glycerol 55	0.3237	def
control no 0	0.3450	defg
raw glycerol 45	0.3508	defg
raw sorbitol 35	0.3771	efg
raw sorbitol 45	0.3839	fg
AAT sorbitol 45	0.4163	g
HTT glycerol 55	0.5322	h
raw sorbitol 55	0.5526	h

Analysis of variance

Variate: retinol retention in day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	0.3345	0.1115	0.98	0.407
plasticizers	2	0.1537	0.0769	0.67	0.513
concn	3	1.3530	0.4510	3.95	0.011
treatment.plasticizers	1	2.1746	2.1746		<.001
treatment.concn	3	0.7720	0.2573	2.25	0.087
plasticizers.concn	1	0.1115	0.1115	0.98	0.326
treatment.plasticizers.concn	5	1.2114	0.2423	2.12	0.070
Residual	95	10.8526	0.1142		
Total	113	16.9632			

Fisher's unprotected least significant difference test

Variate: retinol retention in day 2

Treatment: plasticizers.concn

Treaments	Mean	S. D. Indicator
raw glycerol 35	0.610	a
HTT sorbitol 35	0.751	ab
HTT sorbitol 55	1.057	bc
control no 0	1.094	bc
HTT glycerol 35	1.120	bc
HTT glycerol 45	1.159	c
raw glycerol 55	1.202	c
AAT glycerol 55	1.206	c
raw glycerol 45	1.223	cd
AAT glycerol 45	1.239	cde
AAT sorbitol 35	1.261	cde
HTT sorbitol 45	1.267	cde
AAT sorbitol 55	1.278	cde
AAT glycerol 35	1.282	cde
raw sorbitol 45	1.338	cde
raw sorbitol 35	1.364	cde
AAT sorbitol 45	1.377	cde
HTT glycerol 55	1.593	de
raw sorbitol 55	1.621	e

Analysis of variance

Variate: retinol retention in day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	3	0.85149	0.28383	9.90	<.001
plasticizers	2	0.00657	0.00328	0.11	0.892
concn	3	1.35293	0.45098	15.73	<.001
treatment.plasticizers	1	3.53928	3.53928	123.42	<.001
treatment.concn	3	0.53139	0.17713	6.18	0.002
plasticizers.concn	1	0.51899	0.51899	18.10	<.001
treatment.plasticizers.concn	5	2.40316	0.48063	16.76	<.001
Residual	38	1.08973	0.02868		
Total	56	10.29354			

Fisher's unprotected least significant difference test

Variate: retinol retention in day 3

Treatment: plasticizers.concn

Treatments	Mean	S. D. Indicator
HTT sorbitol 35	0.051	a
raw glycerol 35	0.520	b
HTT sorbitol 55	0.534	b
HTT glycerol 45	0.738	bc
AAT glycerol 45	0.910	cd
raw glycerol 55	0.927	cd
AAT sorbitol 35	0.995	cde
AAT glycerol 35	1.012	cdef
AAT sorbitol 55	1.013	cdef
HTT sorbitol 45	1.047	def
HTT glycerol 35	1.078	def
AAT glycerol 55	1.079	def
control no 0	1.150	defg
raw glycerol 45	1.169	defg
raw sorbitol 35	1.257	efg
raw sorbitol 45	1.280	fg
AAT sorbitol 45	1.388	g
HTT glycerol 55	1.774	h
raw sorbitol 55	1.842	h

Analysis of variance

Variate: β carotene retention in day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.192109	0.064036	14.42	<.001
Plasticizers	2	0.056028	0.028014	6.31	0.004
concn	3	0.059803	0.019934	4.49	0.009
Treatment.Plasticizers	1	0.042754	0.042754	9.63	0.004
Treatment.concn	3	0.091444	0.030481	6.86	<.001
Plasticizers.concn	1	0.092708	0.092708	20.87	<.001
Treatment.Plasticizers.concn	5	0.115129	0.023026	5.18	0.001
Residual	38	0.168769	0.004441		
Total	56	0.818744			

Fisher's unprotected least significant difference test

Variate: β carotene retention in day 2

Treatment: Plasticizers.concn

Treatment	Mean	S. D. Indicator
raw glycerol 35	0.3505	a
control no 0	0.5188	b
HTT glycerol 35	0.5814	bc
raw glycerol 45	0.6382	cd
AAT glycerol 55	0.6661	cde
AAT sorbitol 45	0.6835	cdef
raw sorbitol 45	0.6981	def
raw sorbitol 55	0.6999	def
HTT glycerol 55	0.7065	def
HTT sorbitol 35	0.7255	def
raw sorbitol 35	0.7354	def
raw glycerol 55	0.7389	def
HTT sorbitol 45	0.7435	def
AAT sorbitol 35	0.7639	ef
AAT sorbitol 55	0.7714	ef
AAT glycerol 35	0.7756	ef
AAT glycerol 45	0.7843	f
HTT glycerol 45	0.7896	f
HTT sorbitol 55	0.7896	f

Variate: β carotene retention in day 3

Analysis of variance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	0.212872	0.070957	9.90	<.001
Plasticizers	2	0.001642	0.000821	0.11	0.892
concn	3	0.338232	0.112744	15.73	<.001
Treatment.Plasticizers	1	0.884820	0.884820	123.42	<.001
Treatment.concn	3	0.132847	0.044282	6.18	0.002
Plasticizers.concn	1	0.129748	0.129748	18.10	<.001
Treatment.Plasticizers.concn	5	0.600790	0.120158	16.76	<.001
Residual	38	0.272433	0.007169		
Total	56	2.573385			

Variate: β carotene retention in day 3

Fisher's unprotected least significant difference test

Treatment: Plasticizers.concn

Treatment	Mean	S. D. Indicator
HTT sorbitol 35	0.0255	a
raw glycerol 35	0.2599	b
HTT sorbitol 55	0.2671	b
HTT glycerol 45	0.3691	bc
AAT glycerol 45	0.4552	cd
raw glycerol 55	0.4635	cd
AAT sorbitol 35	0.4974	cde
AAT glycerol 35	0.5062	cdef
AAT sorbitol 55	0.5063	cdef
HTT sorbitol 45	0.5237	def
HTT glycerol 35	0.5388	def
AAT glycerol 55	0.5394	def
control no 0	0.5750	defg
raw glycerol 45	0.5847	defg
raw sorbitol 35	0.6285	efg
raw sorbitol 45	0.6398	fg
AAT sorbitol 45	0.6938	g
HTT glycerol 55	0.8870	h
raw sorbitol 55	0.9210	h

Color Plates

Plate. 1 Film prepared from waste potato starch

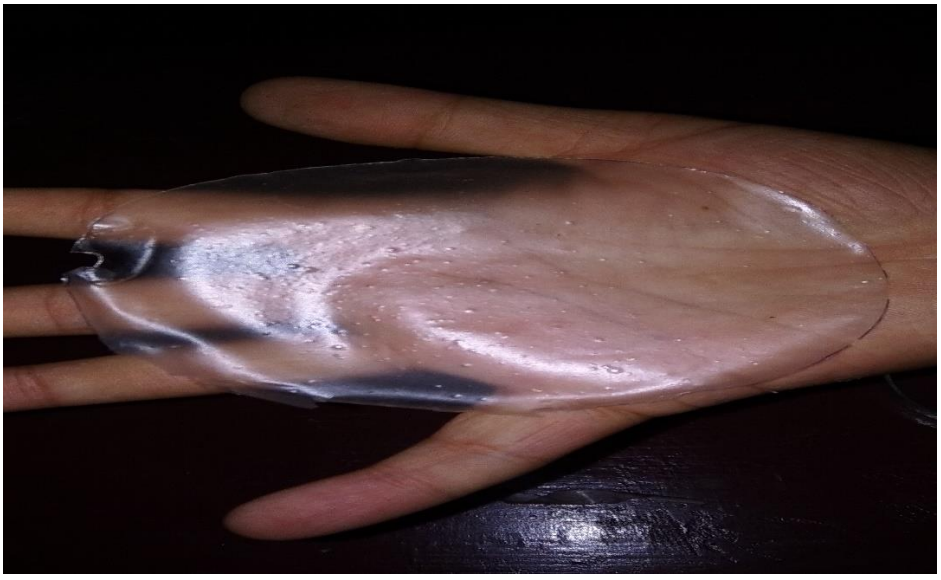

Plate. 2 Coating in fruit

Plate. 3 Apparatus up for measuring Rate of respiration

Plate. 4 Extracted waste potato starch