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Nutritional Modifications and Biochemical Changes during Ripening of Wild Pear (*Pyrus pashia*)

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ABSTRACT
Investigation on the biochemical changes of underlying mechanisms regulating fruit ripening process in *Pyrus pashia* , a wild edible fruiting species from Rudraprayag , Uttarakhand, was carried out. The fruits were collected in different maturity stages; unripe, semi-ripe, and fully ripe and analysed for their morphological and biochemical parameters, flavonoid and phenol contents using standard AOAC methods. It was found that the fruit skin color and specific gravity values can be used to differentiate the maturity stages. Ripe fruits were found to be nutritionally rich , but the unripe fruits had more of medicinal rich properties. This study shall help to provide information about correct harvesting and post-harvest storage period of the fruits required for **optimizing their nutritional value, enhancing shelf life, and exploring their potential for value-added products**. It will also promote commercial utilization of this underutilized fruit species, benefiting local communities and fostering sustainable livelihood opportunities.

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KEYWORDS: Ripening, Maturity, Nutritional, Phytochemical, Post-harvest, Storage

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INTRODUCTION

Pyrus pashia commonly known as the Indian wild pear or *kainth*, is an underutilized fruit species belonging to the family Rosaceae. It is found widely distributed in the subtropical regions of the Himalayas and bears small and oval shaped crown, attractive white flowers with red anthers during February-April and small pear like fruits from May to December. The fully ripe fruit is sweet, has a reasonable flavor and very pleasant to eat when slightly decaying. These fruits are rich source of carbohydrates, proteins, fats, and dietary fiber, providing essential energy and health benefits. Parts of the plant, including fruits, leaves, flowers and bark, are traditionally used for treating digestive disorders, sore throats, skin ailments, used as tonic and sedative. The fruit juice is consumed as a beverage, while the leaves are sometimes used to prepare non-fermented drinks.

Despite its potential, *Pyrus pashia* remains underutilized, with limited research on standardized maturity indices, genetic variations affecting fruit quality, and value addition strategies. The ripening process is critical to the development of fruit quality, which is major contributing factor of consumer acceptance (Prasanna *et al*., 2007; Zhang *et al*., 2022; Pico *et al*., 2022). The stage of maturity impacts fruit quality and commercial value. Studies are needed to develop robust parameters for assessing maturity, improve post-harvest technologies to reduce perishability, and explore innovative processing methods to promote kainth fruits commercialization and sustainable use (Rawat *et al*., 2018). Maturity stages in *Pyrus pashia* fruits significantly influence their biochemical composition, sensory attributes, and nutritional quality. Hence this study was undertaken to quantify the morphological and biochemical changes during fruit maturation, so as to provide insights into the ripening process, optimal harvesting time and potential post-harvest applications.

MATERIALS AND METHODS

All the reagents used for performing the experiments were from Merck.

Sample collection and Processing

Kainth fruit samples of superior *Pyrus pashia* trees were collected from Kameda, Rudraprayag Forest Division, Uttarakhand, during January 2024 (Figure 1). Samples were categorized into three maturity stages using visual parameters: unripe, semi-ripe and ripe

(Figure 2). These were washed and stored in deep freezer. For biochemical analysis these were taken out, dried in oven, powdered and then analysed according to the following protocols:

Nutritional Analysis

Standard AOAC protocols were used (2002). Morphological parameters were measured using vernier calipers (Mitutoyo Make). Moisture content was determined by oven drying method. Specific gravity was determined by water displacement method and fruit color was evaluated using RHS (Royal Horticulture Society) color codes. Total Soluble Solids (TSS) were determined according to the method described by Mazumdar and Majumder (2003) using hand held refractometer (range 0–32%). An appropriate quantity of each sample was placed on the prism-plate of the refractometer and the reading appearing on the screen was directly recorded as total soluble solids (°Brix). Titrable Acidity was determined according to the method described by Hortwitz (1960).

Ash content was determined using muffle furnace; fat content through Soxhlet extraction method; crude protein (CP) content by the Kjeldahl method. Nitrogen content was multiplied by the factor 6.25 to determine the CP. Vitamin C was determined using Indophenol method (Sadasivam & Balasubramaniam, 1987) The amount of vitamin C in the extract was determined by comparing with the titration curve of standard vitamin C solution. Result was expressed in mg/100 g of fresh fruit.

Biochemical Analysis

Aluminum trichloride complex assay (absorbance at 415 nm) was used to determine total flavonoid content (Markham, 1982). Total phenolic content was determined using Folin-Ciocalteu reagent assay (absorbance at 740 nm) (Peñarrieta *et al*. 2007**)**.

RESULTS AND DISCUSSION

Fruit maturation is a dynamic and complex process characterized by various biochemical transformations that significantly affects fruit characteristics such as taste, texture, color and aroma. These changes are essential for the fruit to become palatable, nutritious, and appealing to consumers. In *Pyrus pashia*, the ripening period can extend from May to November, with some fruits continuing to mature upto late December or even January in colder regions. Mature fruits are dark brown in colour, soft and perishable.

During the transition phase from growth to ripening, fruits experience an increase in respiration rate, commonly referred to as the climacteric rise. This transition involves an increase in glycolysis, the citric acid cycle, and the production of ethylene, a plant hormone that regulates ripening. Ethylene induces a series of metabolic changes, such as the breakdown of starches into sugars, which contribute to the sweetening of the fruit (Paul & Pandey, 2014). *Pyrus pashia* fruit is seen ripening on trees and is therefore a climacteric fruit.

Color development is an important biochemical process during fruit ripening. The degradation of chlorophyll and the synthesis of pigments such as carotenoids, anthocyanins and other compounds lead to the distinctive color changes (Kapoor *et al*, 2022). These colour changes are vital for visual appeal and serve as indicators that the fruit is ready for consumption. During *kainth* fruit maturing, color changes were observed. The early fruit is mostly of light green color but at maturity, its color turns blackish brown. The skin transitions from medium yellow to yellow-orange in partially ripe fruits, ultimately turning brown when fully ripe, with juice color varying in shades of brown were observed as per the RHS color chart (Table 1).

Table 1: Colour changes in *Kainth* fruit during various maturity stages

Fruit stages of *P.pashia*	**Skin colour**	**Juice colour**
Unripe	V-Yellow (No. 4A med. Yellow)	III Red (No. 175 A, Medium Brown)
Partially Ripe	V-Yellow (No. 16C med. Yellow Orange)	III Red (No. 200C Dark Brown)
Fully Ripe	III Red (No. 200A, Dark Brown)	III Red (No. 175 C, Medium Brown)

Pyrus pashia ripe fruits exhibit higher moisture content (56.45%) compared to unripe (52.84%) fruits, indicating formation of juice in fruits as they mature. Fruit dimensions (length and width) and weight decrease significantly as ripening occurs with a 38.37% reduction in weight observed during the ripening process. Specific gravity (SG) increases in ripe fruits, causing them to float compared to unripe fruits which sink in water (Table 2). Hence this SG method can be used to identify ripe fruits easily.

Table 2: Morphological changes in *Kainth* fruits during various maturity stages

Parameter (%)	Unripe	Partially Ripe	Ripe
Moisture content %	52.84 ± 1.60	53.88 ± 1.55	56.45 ± 2.68
Fruit length (mm)	18.70 ± 0.63	18.25 ± 0.71	16.28 ± 0.33
Fruit width (mm)	20.55 ± 0.62	19.82 ± 0.25	17.93 ± 0.22
Fruit weight (gm)	6.02 ± 0.17	4.85 ± 0.19	3.71 ± 0.41
Specific gravity	0.83 ± 0.08	0.86 ± 0.08	1.02 ± 0.06

Lipid hydrolysis is catalysed by lipases. During ripening phase this mechanism may play role in development of volatile chemicals that give foods their distinctive scents as well as changes in texture and flavor (Panzanaro , 2010). Fat content increased with ripening from 0.52 to 0.83 % in *kainth* fruits.

The content of crude fiber in fruits is inversely proportional to carbohydrate and sugar levels. This inverse relationship results from the degradation of polysaccharides during ripening (Fitriningrum *et al*. 2013). Ash content decreases as *kainth* fruit matures. These observations are consistent with findings of Haq (2006) , Jagdeesh *et al* (2010) and Chandra and Bharti (2020). Decrease in mineral content with the progression of ripening is likely due to the utilization of minerals during fruit growth.

Carbohydrates are fundamental in shaping the colour, flavor, taste and texture of mature fruits. During maturation, starches stored in the fruit are converted into simpler sugars, such as glucose, fructose, and sucrose. Enzymes such as amylase, invertase and sucrose synthase play crucial roles in this conversion. The shift from starch to sugars is a major contributor to the sweet flavor of ripe fruits, as seen in bananas, grapes, and peaches (Elhadi *et al*., 2019). Consistent with previous studies, the carbohydrate content decreases during ripening, resulting in the enhanced sweetness of fully ripe fruit (Marc, *et al*., 2024). Total soluble sugars of fruits is a major quality parameter, which is correlated to the texture and composition. Total soluble sugars (TSS) increased from 9.64 % in unripe to 15.16 % in ripe *kainth* fruits.

Protein synthesis primarily occurs during the early stages of ripening, prior to significant physical changes, such as color transformation. In *Pyrus pashia* the highest protein content was observed in mature fruits during the ripening stage (Table 2). Tlili *et al*. (2014), have reported increases in protein content in *Rhus tripartitum* fruits as they progressed from immature to intermediate stages. However, protein content decreased significantly in *Rhus tripartitum* fruits after the intermediate stage, dropping by over 25%. Although this reduction does not typically affect the nutritional value, it may influence the flavor profile of the fruit. Our results however, align with the work of Blakey *et al.* (2012 & 2014) on ripening of Avocado. The rise in protein levels during ripening may be attributed to the activation of enzymes like cellulase and polygalacturonase, which aid in fruit softening (Giribaldi *et al*., 2007).

Acidity plays a significant role in the flavor profile of fruits. As ripening progresses, the concentration of organic acids, such as citric acid, malic acid, and tartaric acid, typically decreases. This decrease can be seen in ripe *Pyrus pashia* fruits. This reduction occurs through the action of enzymes like polygalacturonase, pectinase, and acid phosphatase. The decline in acidity, combined with the increase in sugar content, contributes to a sweeter and more palatable taste. Furthermore, the decrease in acidity alters the pH of the fruit, making it less tart and more appealing for consumption. The vitamin C content of several fruits is known to be controlled during fruit growth. The data from two experimental years showed that the significantly highest content of vitamin C was in *Solanum melanocerasum* fruits and ranged from 48.15 mg 100 g^{-1} at ripening stage I to 45.10 mg 100 g^{-1} at ripening stage III (Staveckienė *et al*, 2024). Our results with *Pyrus pashia* fruits showed increase in Vitamin C content during fruit ripening. Results of variation in nutritional parameters are depicted in Table 3.

Table 3: Nutritional value of *Kainth* fruits in different maturity stages

Parameter (%)	Unripe	Partially Ripe	Ripe
Crude Fat	0.52 ± 0.04	0.68 ± 0.03	0.83 ± 0.12
Crude Fibre	43.37 ± 1.66	41.52 ± 1.03	38.26 ± 1.27
Total Ash	0.97 ± 0.02	1.48 ± 0.07	1.93 ± 0.25

Carbohydrate	40.85 ± 1.59	40.68 ± 1.26	39.07 ± 1.88
TSS	9.64 ± 0.40	11.34 ± 0.47	15.16 ± 1.05
Crude Protein	2.93 ± 0.08	3.18 ± 0.30	4.08 ± 0.12
Titrable acidity	0.71 ± 0.035	0.53 ± 0.04	0.45 ± 0.03
Vitamin C (mg/100g)	2.36 ± 0.15	3.23 ± 0.25	4.06 ± 0.23

Phenols and flavonoids are one of the most vital bioactive compounds, the composition and content of which defines the quality and flavour characteristics of fruits. In recent times, they have received greater attention due to their inherent antioxidant capacities with remarkable health benefits in prevention of various oxidative stress associated diseases, *viz*. cancer, cardiovascular diseases etc. (Dai and Mumper, 2010). During fruit ripening there is a gradual change in certain secondary metabolites with the commencement of phenolic pathways. The newly synthesized phenolic compounds play a crucial role in the fruit pigmentation, as well as its pathogen resistance (Ghasemzadeh and Ghasemzadeh, 2011). The content accumulation in fruit and vegetables is largely affected by genotype, cultural practice, pre-harvest environmental conditions, degree of maturity at harvest, storage conditions and processing techniques (Shahidi and Naczk, 2003). The phenolic content of most fruits declines from early growth stages till attainment of physiological maturity. Significant variations were observed in flavonoid and phenolic content across maturity stages in *Pyrus pashia* fruits, with unripe fruits showing superior medicinal properties (Table 4).

Table 4: Phytochemical variation in fruits in different maturity stages

Parameter	Unripe	Partially Ripe	Ripe
TFC (mg/g QE)	29.14 ± 1.89	23.45 ± 1.96	17.87 ± 3.02
TPC (mg/g GA)	26.59 ± 0.57	17.72 ± 0.57	14.36 ± 1.13

Currently underexploited, *Pyrus pashia* fruits offer substantial commercial potential. The biochemical changes that occur during fruit maturation are pivotal in determining the quality and sensory attributes of fruits. Understanding these processes not only enhances our ability to optimize fruit production and quality but also provides insights into how fruits can be harvested and processed for maximum consumer appeal. Future research in this area can focus on the molecular mechanisms underlying these biochemical changes, with potential applications in agriculture, food science and biotechnology.

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AUTHORS' CONTRIBUTIONS

Conceptualization & designing of the experiments (MR); Execution of field/lab experiments and data collection (MG, SS, PK,MR) and preparation of the original manuscript (MR) ; Analysis of data and interpretation (MR); Editing of the manuscript (MR)

DECLARATION

The authors declare that they have no conflict of interest.

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Tree	**Flowering**	**Profuse Fruiting**

Figure 1: *Pyrus pashia* tree in Natural Habitat

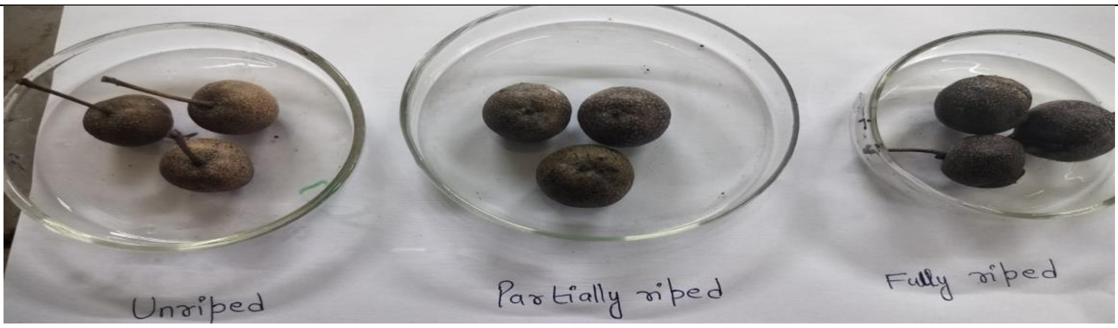

Figure 2: Different stages of maturity in *Pyrus pashia* fruits