Changes in activity of polygalacturonase (PG), pectinesterase (PE) and cellulase (Cx) in pulp during fruit development of two ripening Jujube Ziziphus spp. cultivars

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Abstract. The activities of polygalacturonase (PG), pectinesterase (PE) and cellulase (Cx) were investigated in two jujube cultivars (Zatoni and Bambawi) during fruit development. The jujube fruit Zatwni and Bambawi cvs, displayed a double sigmoidal growth curve with very short stage II (2 weeks in Zatwni and 4 weeks in Bambawi cv.). the pectin content was high during early stage of fruit development, but declined gradually with fruit development and reached their lowest value when the fruit entered the ripening phase. The PG, PE and Cx were low level during early stages of fruit development, but there were rapid increase in these enzymes activity as the jujube entered variation stage, and maturity phase.

1. Introduction
Ziziphus spp. have many common names known as Jujube, Chinee Apple, Jujube, Indian plum, Masau and in (Arabic) : Nabiq or sidr (fruit). Sidr is a tropical fruit tree species belonging to the family Rhamnaceae. Jujube fruit is important fruit in tropical and subtropical areas which is grown all over the drier parts of the Indian subcontinent, Africa and northern Australia for its fresh fruits. It is a particularly good tree to grow in dry regions, because it can withstand long periods of drought. It has a long taproot and can withstand high temperatures during the summer. Therefore, it is found as a wide spread fruit tree in southern Iraq, and comes second crops after date palm in the annual fruit population [1;2]

Ripening is the physiological and chemical process in fruits that causes them to become more palatable. In general, a fruit becomes sweeter, less green, and softer as it ripens [3;4]

Fruit ripening is a highly coordinated, genetically programmed, and an irreversible phenomenon involving a series of physiological, biochemical, and organoleptic changes, that finally leads to the development of a soft edible ripe fruit with desirable quality attributes. Excessive textural softening during ripening leads to adverse effects/spoilage upon storage. Cell walls play a key role in cell protection and in the regulation of intercellular exchanges then play important changes in fruit ripping, Enzymes which play a key role in chemical
ripening changes, and the most important cell wall degradation enzymes are Pectinesterase (PE), Polygalacturonase (PG), Cellulase (Cx) and many other enzymes [4].

Pectinesterase (PE) is an enzyme widely found in plants and microorganisms, which hydrolyses the methyl ester bonds of the esterified carboxyls, releases methanol and transforms the pectin in low methoxyl pectin and even polygalacturonic acid. PE has been extracted and characterized as part of the ripening behaviour of kiwi [5], pear[6] and guava [7]. It has been proposed that the control of the enzymatic action will control the process of softening, which will result in an increase of the commercialization of the fruits and a better profit and rateability of the fruits.

During fruit ripening, PG is mainly responsible in dissolution of the middle Lamella. Exo PG breaks down pectin by hydrolysing the α-1,4-glycosidic bonds between the galacturonic acid residues in galacturonans from the non-reducing end, which results in the release of galacturonic acid as the major reaction product[8]. Also during the ripening of some fruits cellulases are produced that break down the cellulose in cell walls causing softening. All these enzymes contribute to the increased of the softness of the fruit and then ripening, the changes in these enzymes during growth and ripening of fruit have been studied by many researchers, in Tomato [9]. Pear [10], date palm [4], the physiological study of the cell wall fruit enzymes are very important to explain the chemical changes in fruit and gave a general look to ripening date therefor this paper highlight study cell wall enzymes in the sider fruits which is one of the few studies in this area.

2. Materials and Methods

The present study aims to study the physiological changing in cell wall enzyme activity of fruit by selected two cultivars ‘Zatwni and Bambawi’ five-year-old jujube plants, were grown in the Jujube private field at the Al-Hartha region - Basrah-IRAQ.

2.1. Sampling collection and preparing

The samples were collected from 6 plants of each cultivars ‘Zatwni and Bambawi’ which were planted with a row spacing of 4 m × 3m, all of the plants were grown in accordance with local management standards, including pruning, fertilizing and pest control. Samples were collected once every 15 days after the fruit set stage until the fruits ripened. The fruits were considered ripe when the skin colour changed from green to light red. Five fruits were collected at each sampling. The samples were randomly collected from the periphery of the upper canopy and the upper part of the fruit-bearing branches of trees with similar growth, and the fleshy tissue of the 5 fruits was cut into small pieces and mixed together. Approximately 10 g of each sample was placed in a freezing vial and stored at -5°C until use [4].

2.2. Enzymes assay

Extracted and estimated of plant enzyme (PE, PG, Cx) describe in [4]

2.3. Fruit physiological characters

Samples of 40 fruits, 20 fruits were taken randomly from each tree (each 2 trees is replicate) to determine fruit weight.

2.4. Pectin content:

Pectin was determined according to [11] .

2.5. Statistical analysis:

The statistical analysis of the data was done using the general liner model (GLM) and analysis of variance (ANOVA) technique. The mean of 3 replicates for each period for each cultivar were compared by the least significant difference (LSD 5%). The statistical analysis was performed with the help of statistical software package [12].
3. Results and Discussions

3.1. Fruit growth curve:
The growth curve of the two jujube cv. (Zatwni and Bambawi) was shown in Figure 1. The growth curve is a double sigmoid growth curve with three growth phases. Stage I is a period of rapid weight growth. This period requires approximately 8 weeks. Then the fruits entered into the lag phase of growth, which lasted for 2 weeks in cv. Zatwni and 4 weeks in cv. Bambawi. Then the fruit enters into stage 3th, the end of this stage is maturation. The weight of fruit resumed rapid growth rate and this stage lasted for 6 and 8 weeks in Zatwni and Bambawi respectively, at the end of this stage the fruit was interred in maturity then ripening.

![Figure 1](image1.png)

**Figure 1.** Changes in fruit weight during fruit growth and development of Zatwni and Bambawi cultivars

3.2. Pectin content:
Figure 2 showed changes in pectin content of fruit during development of the two cultivars jujube fruit (Zatwni and Bambawi). Maximum pectin content recorded in 6 weeks after fruit set of 2.9% and 2.6% of in cvs. Zatwni and Bambawi respectively. Pectin content (Figure. 2) also showed continuous decline from first stage to the end of second stage. Then the pectin was rather stable at the beginning of the third stage between 10 and 12 weeks, then the pectin content resumed rapid decline till the fruit ripening.

![Figure 2](image2.png)

**Figure 2.** Changes in pectin content during fruit growth and development of Zatwni and Bambawi cultivars

3.3. Changes enzymes activity
The PG activation slightly increased till 8 and 10 weeks after fruit set in both two cultivars Zatwni and Bambawi respectively, then sharply increased, reaching the peak at 12 and 14 weeks after fruit set (199 and 148 microequivalent / kg/ min respectively), which coincided with the variation of fruit colour (Figure. 3).
Figure 4. Shows changed in PE enzyme activity (microequivalent / kg/ min) during fruit development of two jujube cultivars, Zatwni and Bambawi. The activity of PE was absent and not detectable at first the stage of fruit development then the PE activity was a little low and increased along with fruit development up end of stage II. It the obvious that activity was low during the first 8 weeks of fruit development from fruit set, but there was a rapid increase in this activation entered venison at 14th weeks which reached (56 and 55 microequivalent / kg/ min respectively). Then the PE activation declined till the end of fruit ripening.

Figure 3. Changes polygalacturonase activity during fruit growth and development of Zatwni and Bambawi cultivars

Figure 4. Changes Pectinesterase activity during fruit growth and development of Zatwni and Bambawi cultivars

Cellulase activity increased gradually along with fruit development of the two jujube cultivars. It rises dramatically from 2 to 4 weeks after fruit set and from 12 to 14 weeks after fruit set for Zatwni and Bambawi respectively (Figure 5.).
Figure 5. Changes Cellulase activity during fruit growth and development of Zatwni and Bambawi Ber

The first growth stage may be cause by cell division and cell enlargement. Several studies such as [1;2] found there was higher level of promotion hormones (IAA, GA3 and CY) in this stage therefore this stage is active in cell division and cell enlargement. But when the fruit in second stage was the fruit intend in depressed period of growth, the physiological basis of this depressed phase remains obscure but [1] and [3;4] suggested the low level of promotions (IAA, GA3 and CY) may be the cause of this depressed growth rate. Then when fruit growth carve entered in stage three the frit was resumed to raped growth, the rapid growth rate of this stage due to high move of water and nutrition (sugar) from other parts of plant to fruit [3]. Figure .2. showed pectin content decreased with the advancement of ripening in both cultivars. the rapid decline of pectin content was due to the increased activity of cell wall enzymes PE and PG (Figure 3, 4), which were works on pectin, this result was reported by other researcher [6]. The activity of PG in the first of stage III, may be due to climacteric peak of respiration and Ethylene [1], this is a signal that chemical and fruit firmness was started and then subsequently decreased thereafter, from previous resulted the relationship between cell wall enzymes and fruit ripening, the relationship of compatibility between Polygalactronic PG and pectin esterase PE, as the enzyme PE active in the early green stages to remove methoxy groups found in pectin chains which hinder the work of the enzyme endo-PG [13], which was missing during the first stage. The effectiveness of both enzymes present in the yellow phase. Unlike the green phase, it has been observed in the maturity stage that PG enzyme activity rise to the peak. After that the activity of two enzymes decline when ripening [14]. Therefore we can say that the enzyme PE has an indirect role in firmness and maturity of fruit, while the PG enzyme has a direct role in it. This compatibility between PE work and PG in fruit is controlled by genetic programming of the fruit [4], means when the fruit reaches the suitable age, some genes are activated then send the signal from nuclear to cytoplasm to promote the syntheses of some proteins or enzymes which contribute to the ripening processor. The cellulose activity of enzyme at the first stage III but had no significant level between the two cultivar fruits. It is possible that enzyme has a role in ripening processes, perhaps in conjunction with polygalacturonase [10], to work fruit firmness, and [9] suggested cellulose may act in tomato fruit in conjunction with pectin enzymes to cause softening.
4. Conclusions
We noted in all figures, the activity of cell wall enzymes in early cultivar Zatwni precede the late cv. Bambawi, this may contribute in fruit ripening of Zatwni cv. which is more early comparative with Bambawi cv. or beside the inheritance treats, the PG and Cellulase enzymes play a major role in ripening of jujube fruit.

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