RESEARCH PAPER
J. Food Sci. & Technol. Nepal, Vol. 7 (31-35), 2012
ISSN: 1816-0727

Proteins Play Important Role in Intercellular Adhesion Affecting on Fruit Textural Quality

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Fruit textural quality is becoming a major quality parameter for export, postharvest preservation, handling and processing. The main determinant of textural quality is intercellular adhesion (ICA) as attributed by the cell wall (CW) and its components. The importance of CW protein in ICA strengthening was exemplified in Medjoul date (Phoenix dactylifera L.) fruit, as a model. Fruit mesocarp sensitively responded to culture environment which was assayed in vitro at pH 3.5(< pKa) and pH 6.5(> pKa) in presence of organic acid molecules. The max penetration force, as a measure of ICA strength, of pH 3.5 (< pKa) incubated mesocarp (~10.5 N) was significantly higher than that of pH 6.5 (> pKa) incubated fruits (~2 N). The protein bands at ~29 kDa, ~75 kDa, ~32 kDa and 87 kDa were exclusively or prominently found in ICA strengthened fruits (pH 3.5< pKa) compared to texturally injured fruits (pH 6.5 > pKa).

Keywords: Cell wall, Protein, Intercellular adhesion, Date fruit

Introduction

The quality requirements of the export fruits have become less and less compromising. The chemical and physical characteristics of the fruits influence their mechanical and rheological properties and ultimately of textural quality (Shomer *et al.*, 1998; Ismail *et al.*, 2006). These attributes are particularly of interest at quality control in sorting, grading, handling, packaging and storage of fruits and their products. However, the most important quality factor is the texture as related with the mouth senses, freshness feeling and attractiveness for consumption.

The change in the textural quality of the fruit depends upon the loosening or the intactness of the interconnection among the large thin walled parenchymatous mesocarp cells and its consequences on exocarp (Hass and Bliss, 1935; Rygg, 1946; Hasegawa *et al.*, 1972; Shomer and Kaaber, 2006). Towards understanding of the quality determinants, it is crucial to identify factor(s) conferring the fruits capabilities to withstand textural injuries. Therefore, it is of basic importance to study the apoplast or intercellular adhesion (ICA) strength as related to the composition of the primary cell wall (CW) and the middle lamella and their associated agents such as hemicelluloses, neutral sugars, proteins and ions. Generally, CW is the apoplast skeleton playing role in texture of plant parenchyma particularly where compartments such as secondary lignin assembly or non-CW composites such as protoplasmic insoluble storage proteins, oils/fats and starch are residual or absent (Vian, 1982; Varner and Lin, 1989; Shomer and Kaaber, 2006; Cantu *et al.*, 2008a; Cantu *et al.*, 2008b; Caffall and Mohnen, 2009).

Pectin metabolism remains the most studied aspect of CW biology in most of the fruits and usually, ICA strengthening is attributed by Ca-pectate (Jarvis, 1984; Shomer *et al.*, 1984; Goldberg *et al.*, 1996; Cosgrove, 2000; McCartney and Knox 2002; Jamet *et al.*, 2006; Iwai *et al.*, 2006; Vicente *et al.*, 2007; Popper and Fry, 2008). However, recent study (Shomer and Kaaber, 2006) has raised the questionable role of Ca-pectate in textural damage which highly emphasized on CW embedded specific proteins that play important role in ICA. The expression level and the activities of different enzymes like polygalacturonases (PGs), pectin methylesterase (PME), arabinogalactan protein (AGP), cellulase, polyphenol oxidase, β-galactosidase, α-arabinosidase, etc. are of particular interest to fruit quality since they change CW structure and composition which consequently determines textural behavior (Kramer *et al.*, 1993; Showalter, 1993; El-Zoghbi, 1994; Ahmed *et al.*, 1995; Zhang *et al.*, 1996; Stolle-Smits *et al.*, 1999; Vogel *et al.*, 2002; Jarvis *et al.*, 2003; Cho *et al.*, 2005; Brummell, 2006b; Cantu *et al.*, 2008b; Osorio *et al.*. 2008). In this study, date fruit (*Phoenix dactylifera* L.) was used as a model. CW proteins in texturally injured (CW separated from the mesocarp) and texturally superior (CW well adhered to the mesocarp) fruits were extracted and analyzed.

Materials and Methods

Plant material- Date (*Phoenix dactylifera* L.) fruits of Medjoul variety were investigated in relation to their texture and textural damages. Young green fruits were collected from orchards in the Northern Jordan Valley area and Dead Sea Area.

Chemicals- Most of the chemicals used in this study were purchased either from Sigma-Aldrich, USA or Merck, Germany.

Induction of ICA strengthening- Date (*Phoenix dactylifera* L.) fruits of Medjoul variety were investigated as a model in relation to their textural behavior. The induction of ICA strengthening or loosening is based upon the procedures of

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Shomer and Kaaber (2006). Cross-sectioned slices (~1 cm thickness) of young developing green date fruits (~3 cm dia.) were incubated for 7 to 11 days upon solidified agar containing 50 mM formic (HCOOH) or acetic (CH_3COOH) or propionic (CH_3CH_2COOH) acid buffers at either pH 3.5 < pKa, pH = pKa (3.75, 4.75 and 4.87, respectively) or pH 6.5 > pKa at 20 °C.

Analysis of ICA strength- ICA/apoplast strength of mesocarp of fresh and incubated fruits' cross slices was analyzed using texture analysis by penetration of 2-mm dia. cylindrical probe in TA-XT2 Texture Analyser (Shomer and Kabber, 2006).

Separation of CW- Mesocarp CW was separated after homogenization of 20 g mesocarp tissue in 60 ml of 25 mM phosphate buffer (pH 7.0) with 2 mM sodium disulfite (Racusen and Foote, 1980; Andrews *et al.*, 1988; Bohac, 1991) by a WARING commercial blender at medium speed. In order to get the crude CW matter the homogenate was vacuum filtered in Cinter Glass (PYREX, England) after extraction.

Separation of CW bound proteins and characterization- CW bound proteins were extracted according to Kim *et al.*, (2001) using glass powder and Mg/NP-40 buffer solution (including 0.5 M Tris HCl, pH 8.3, 2% v/v NP-40, 20 mM $MgCl_2$, 2% v/v β-mercaptoethanol, 1 mm phenylmethyl sulfonyl fluoride and 1 % w/v polyvinylpolypyrrolidone) under liquid nitrogen. CW protein was characterized qualitatively (Laemmli, 1970) in sodium dodecyl sulfate polyacrylamide gel (12%, w/v, 0.75 mm) electrophoresis (SDS-PAGE).

Results and Discussion

This study dealt with textural injury/resistance towards understanding the effect of environment and underlying mechanism determining textural quality in fruits. The texture of the fruit mesocarp sensitively responded to the environmental variations revealing remarkable differences between fruits following incubation at various conditions. It has been again proved and supported that the cell cultures by short chain-/linear-/monocarboxylic acids buffer provides an ideal tool for the study of the role of molecules of cell surface in ICA and cell separation (Shomer and Kaaber, 2006).

Changes in appearance- The appearance of the fruit tissue incubated at pH 3.5 < pKa appeared to be close and compact to each other with exocarp remaining intact with mesocarp (Figure 1).

Figure 1. Appearance of fruits' slices as fresh and following a week of incubation upon 50 mM acetate acetate buffered agar media

Contrarily, the tissues of pH 6.5 > pKa incubated fruit slices appeared to be highly damaged and cracked where the exocarp became loosened/separated (Figure1). Fruit slices incubated at pH = pKa were slightly injured.

Changes in ICA strength- The apoplast strengthening of date fruit cross slices was reliably and repeatedly induced *in vitro* in presence of 50 mM buffer of linear, short carbon chain, monocarboxylic organic acid molecules, i.e. at pH 3.5 < pKa (Figures 1 and 2). The penetration force, as a measure of ICA strength, of pH 3.5 < pKa incubated mesocarp was very similar as in control fresh (Figure 2C) and both of them were significantly higher than that of texture injured mesocarp incubated at pH 6.5 > pKa (Figure 2C). Moreover, max penetration force (~10.5 N) of pH 3.5 < pKa induced fruit was even higher than that of control fresh (~9.5 N) and both of them were significantly higher than that of pH 6.5 > pKa incubated fruits (~2 N). Moreover, pH 3.5 d" pKa incubated mesocarp reached even higher total ICA strength (Figure 2D) than that of fresh non-incubated fruit (60.7 ± 16.5 and 55.2 ± 12 J, respectively). In comparison, the pH 6.5 e" pKa incubated fruit tissue exhibited textural injury with negligible ICA strength (Figures 2C and 2D). The change in ICA directly or indirectly is dependent on an association between organic acid molecules/anions and a defined tissue sites. Because pH changes result in ICA alterations where strengthening is induced at pH < pKa, it seems that the strengthening is induced by the lipophilic acid molecules rather than the pH per se. This finding suggests that ICA strengthening is induced when acid molecules are associated with lipophilic cell compartment(s).

Cell wall proteins- Significant variations are found on SDS-PAGE bands of CW bound proteins between well textured and injured textured date fruits (Figure 3). Most of the CW proteins were ranged in between 26 to 90 kDa with two major bands at ~29 and ~75 kDa which were prominent in ICA induced fruits (pH 3.5 d" pKa) than in texturally injured fruits (pH 6.5 d" pKa). Moreover, proteins at ~32 kDa and 87 kDa were exclusively found only in ICA induced fruits (pH 3.5 d" pKa). Proteins in the range of 37 to 50 also revealed some differences but with poor visibility. Remarkable differences were also found in naturally injured and well textured matured fruits (data not presented). The presence of remarkable exclusive and prominent CW proteins only in well textured fruits clearly defines their role in apoplast/ICA strengthening determining textural behavior of fruits. This has been supported by previous findings that the proteins gathered and cross linked in parenchyma CW involve in conferring apoplast strength consequently determining fruits behavior (Showalter, 1993; Jamet *et al.*, 2006; Shomer and Kaaber, 2006; Cantu *et al.*, 2008b). However, it is necessary to explore such specific proteins whether they are CW invertase, glucanase, ATP synthase, or some others which are related somehow in CW strengthening (Zhang *et al.*, 1996; Cho *et al.*, 2005).

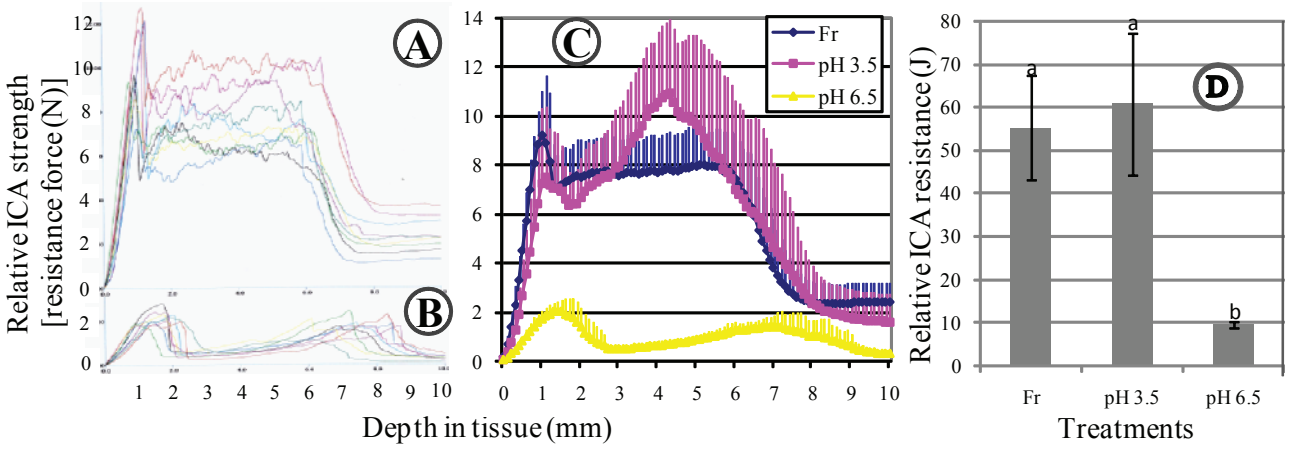

Figure 2. Representative ICA strength of date fruit mesocarp [expressed as resistance force profiles (N) for penetration of 2-mm diameter cylindrical probe], following incubation upon 50mM of acetate buffered agar at pH 3.5 and 6.5 at 20 °C for one week as compared to non-incubated control tissue (Fr). Values are presented as mean + S.D. of eight measurements of 4 repetitions and 2 replicates. The line fresh (Fr) in (C) is the mean of lines in (A) and the line pH 6.5 is the mean lines in (B). (D), average ICA strength [as resistance energy (J)]. Values marked with different letters are significantly different (P d" 0.05)

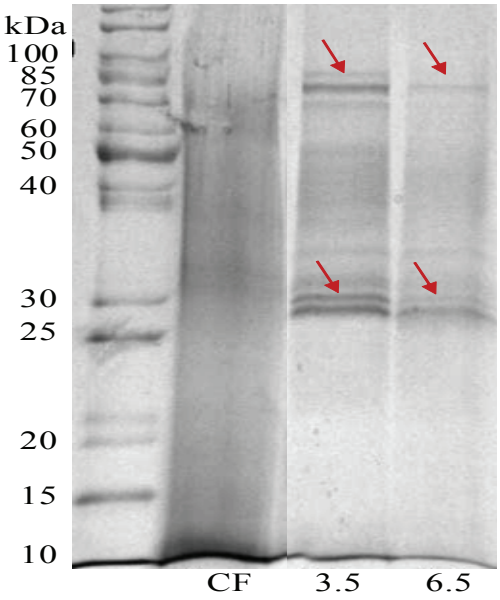

Figure 3. SDS-PAGE of mesocarp CW bound proteins of green date fruits. CF, fresh control; 3.5 and 6.5 *in vitro* incubated upon acetate buffered agar media with pH 3.5 < pKa and pH 6.5 > pKa; Arrows mark specific protein bands prominent in ICA strengthened fruits (pH 3.5 < pKa) and residual or absent in texture injured (ICA loosened) fruits (pH 6.5 < pKa)

While, being enzymes, some proteins such as pectinases (pectin methyl esterase, polygalacturonase, pectinlyases), cellulases, xylanase, etc. influence the apoplast strength in various stages of tissue life (Showalter, 1993; Jamet *et al.*, 2006; Shomer and Kaaber, 2006). Moreover, cysteine proteinase, sialidase, etc. may involve in CW degradation enabling cell separation (Stolle-Smits *et al.*, 1999; Vogel *et al.*, 2002; Jarvis *et al.*, 2003; Grudkowska and Zagdanska, 2004; Brummell, 2006a; Brummell, 2006b; Cantu *et al.*, 2008b; Osorio *et al.*, 2008). This study demonstrates the essentiality of more specific and molecular level study of CW proteins regarding their role in textural behavior of the fruits.

The well established finding is that the alteration in pectin components, especially the Ca-pectate determines the ICA strength and consequently changes the textural quality of the fruit (El-Zoghbi, 1994; Goldberg *et al.*, 1996; Cosgrove, 2000; McCartney and Knox, 2002; Iwai *et al.*, 2006; Jamet *et al.*, 2006; Vicente *et al.*, 2007; Popper and Fry, 2008). But, this study has evidenced that there are some specific CW proteins that plays important role in ICA strengthening and textural behavior. In this study, fruit tissues showed significant response towards the changes in the pH levels of culture condition (Figure 1). Thus, the main cause of the textural alteration is the prevailing environment where the fruit is growing that brings about the gradual changes in CW metabolism which in turn reflects the textural quality of the fruit.

Conclusion

This study has induced textural variations and has elucidated the involvement of proteins in apoplast/ICA strengthening. In conclusion, apart from Ca-pectate, protein deserves as an important component in the ICA strength affecting the fruit's textural quality, which in turn affects consumers' preferences. Moreover, any changes in the protein biosynthesis in agricultural commodities due to agricultural practices, climate changes, storage conditions, processing techniques, etc. may

bring significant alterations in their textural qualities and attractiveness. Our study further necessitates the proteomics study to elaborate the role of specific CW protein affecting textural quality.

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