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Relationships between the textural changes and the contents of calcium, magnesium ions, and non-freezing water in the alcohol-insoluble solids of snap bean pods during cooking processes

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The alcohol-insoluble solids (AIS) of snap bean (*Phaseolus vulgaris* L.) pods after different cooking treatments were used as testing materials. The contents of calcium, magnesium ions and non-freezing water, which were analysed by differential scanning calorimetry, in the AIS were determined and compared with the firmness of the bean pod tissues after different cooking treatments. It was observed that the contents of calcium and magnesium ions in the AIS of the bean pods after direct cooking were lower than those of fresh and precooked bean pods, and were also lower than those of the bean pods after precooking followed by cooking. The contents of these metal ions were significantly and positively correlated to the tissue firmness ($P < 0.01$). The degree of esterification (DE) of pectin in the tissue after precooking was 39.3%. The DE of of pectin in the tissue after precooking followed by cooking was 39.9%. These DE values were significantly lower ($P < 0.05$) than that in the fresh tissue (46.0%) or that in the tissue after direct cooking (46.0%). Moreover, the amounts of non-freezing water in the AIS of fresh and precooked bean pods were larger than those of the bean pods after precooking followed by cooking and direct cooking. This also showed a significant and positive correlation to the tissue firmness ($P < 0.05$). It was apparent that the linkages between the pectin molecules in the tissues of snap bean pods after precooking were changed. This change was caused by the action of pectinesterase in de-esterification of pectin molecules and subsequent formation of calcium or magnesium bridges between the free carboxyl groups of adjacent pectin molecules, which resulted in increases in the amounts of non-freezing water in the AIS and in the tissue firmness. These results can be taken as further supporting evidence for the theory of the firming effect of precooking treatment of vegetables.

INTRODUCTION

Texture, an important attribute of vegetable quality, often varies with the conditions of cooking processes. In general, the texture of vegetables, which are directly cooked at boiling temperature, will be softened. However, if they are precooked first at a moderate temperature (about 50–80°C), and then at boiling temperature, the vegetables will have a firmer texture (Lee *et al.*, 1979; Manabe, 1980; Chang *et al.*, 1986; Tseng & Chang, 1988; Wu & Chang, 1990; Chang & Chang, 1992*a*). This firming effect of precooking was recognised as the result of the action of pectinesterase on the cell wall materials, particularly pectic substances, which resulted in de-esterification of pectin molecules and the subsequent formation of calcium bridges between the free carboxyl groups of adjacent pectin molecules (Hoogzand & Doesburg, 1961; Hsu *et al.*, 1965; Bartolome & Hoff, 1972; Van Buren, 1979; McFeeters *et al.*, 1985; Chang & Chang, 1992*a*).

Pectin is a polymer of D-galacturonic acids linked by α-1,4 glycosidic bonds. The change in the linkages between pectin molecules would affect the interaction of water with pectins and so the state of water in the polymer must be changed. Recently, the interaction of water with polymers has attracted much attention. Watase *et al.* (1988) reported that the water in agarose gels can be classified as free water, freezable disordered

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water, and non-freezing water. Sawayama & Kawabata (1991) also reported that the changes of the amount of non-freezing water caused by the differences between the pectin samples were small, but the effect of the added calcium or magnesium salt was markedly greater. In other words, the addition of calcium or magnesium salt might cause changes of the linkages between pectin molecules, and result in changes of the state of water in the pectin polymer, so that the amount of non-freezing water is changed. In this study, it is intended not only to determine the contents of calcium and magnesium ions in the alcohol-insoluble solids (AIS) of snap bean pods after different cooking treatments, but also to analyse the amounts of non-freezing water in the AIS by differential scanning calorimetry (DSC). This procedure is used in order to facilitate the understanding of the chemical basis of the firming effect of precooking and to reveal further supporting evidence for this effect.

MATERIALS AND METHODS

Materials

Snap bean (*Phaseolus vulgaris* L.) pods were obtained from a local wholesaler. Raw materials of normal size and maturity, and free from decay or mechanical damage, were selected for use. Both ends of the pods were cut off, and only the middle part, about 8 cm in length, was used in the experiment.

Methods

Cooking treatment

The sample, consisting of four pieces of trimmed snap bean pods, was placed in a distilled water bath preheated to a setting temperature, and precooked or cooked for a setting period of time, then rapidly cooled to room temperature. In this study, the conditions for precooking (PC) and cooking (C) were 70°C for 20 min and boiling for 15 min, respectively (Chang & Chang, 1992*a*).

Texture measurement

A rheometer (Model NRM-3002D, Fudoh Kogyo KK. Japan), mounted with a plunger (adapter No. 6), was used to measure the firmness of the samples. The flat base, on which the sample was placed, moved upward to the plunger at a speed of 30 cm/min to measure the maximum puncture forces as an index of firmness of the sample. For each sample, three measurements were taken for each of four pieces of the sample, and the average of 12 measured values was expressed as relative firmness by taking the firmness of the fresh sample as 100.

Preparation of alcohol-insoluble solids (AIS)

The textural changes of most vegetables during processing are mainly affected by the mechanical strength of the cell wall (Van Buren, 1979). In many studies on the compositions of the cell wall of vegetables, the samples were first prepared as alcohol-insoluble solids (AIS) (Manabe, 1980; Lin & Rae, 1982; Loh *et al.*, 1982). The reason for the preparation of AIS was that the changes in the compositions of the cell walls of vegetables, which were caused by the physiological functions, could be inhibited, and this was favourable for analysis. Thus, we used the AIS of snap bean pods after different cooking treatments as raw materials in this study.

Snap bean pod tissue (100–200 g) was homogenised with five volumes of 95% ethanol, and the mixture was kept at 60°C for 40 min to facilitate protein coagulation and subsequent filtration. After cooling to room temperature, the suspension was filtered, and the residue was sequentially washed twice with 80% ethanol and once with diethyl ether, and then dried in an oven at 40°C to obtain the AIS.

Determination of total pectin in the AIS

The total pectin in the AIS of snap bean pods was analysed by using the methods of Ahmed & Labavitch (1977). The AIS (7.5 mg) was digested by 5 ml of concentrated sulfuric acid in an ice bath, then slowly treated with 2.5 ml of de-ionised water and kept for about 1 h. The clear solution of the above digested sample was analysed for its total pectin by using the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) and expressed as anhydrogalacturonic acid (AGA).

Determination of the degree of esterification (DE) of the pectins in the AIS

The DE value of pectin in the AIS of snap bean pods was analysed by the modified method of Klavons & Bennett (1986). The AIS (25 mg) was first saponified with 10 ml of 0.5 N potassium hydroxide at room temperature for 1 h. Then, the saponified solution (5 ml) was adjusted with 0.5 N phosphoric acid to pH 7.5 and diluted with distilled water to make the pectin concentration in the range of 25–200 μg AGA/ml. One ml of the above prepared solution was oxidised with 1 ml of alcohol oxidase (EC 1.1.3.31) (1 unit/ml) at 25°C for 15 min, then added 2 ml of mixed solution of 0.02 M 2,4-pentanedione/2.0 M ammonium acetate/0.05 M acetic acid and kept at 60°C for 15 min. Finally, the absorbance of the solution at 412 nm was determined for its methanol content. The methanol content was multiplied with 31/32, then divided with its total pectin to obtain methoxy content. The DE value of pectin can be obtained by dividing the percentage of methoxy content by 16.32 (theoretical maximum percentage of methoxy content in pectin) and subsequently multiplying by 100.

Determination of calcium and magnesium ions in the AIS

The AIS was wet-digested (Osborne & Voogt, 1978) for determination of calcium and magnesium ions by atomic-absorption spectrophotometry (aa/ae Spectrophotometer, IL151, Instrumentation Laboratory, USA).

Differential scanning calorimetry (DSC)

The DSC measurement was carried out with a Setaram DSC 92 from Setaram Co. (France). The sample of AIS with water (AIS/water = 1/4) was sealed into a stainless crucible and kept at room temperature for over 1 h before determination by DSC. During the DSC analysis, the temperature was lowered to −50°C by liquid nitrogen and maintained for 5 min, then raised at a heating rate of 5°C/min to 50°C. In this manner, the endothermic peak could be measured, and the heat of transition, and the amounts of non-freezing water and freezing water were calculated from the area of the peak. The amount of freezing water was calculated from the endothermic peak of thawing of frozen pure water, and this calculated amount was subtracted from the total water content in the sample to give the amount of non-freezing water (Sawayama & Kawabata, 1991). The value for pure water of 329.3 J/g (shown in Table 2) was used as the latent heat of thawing for ice.

Statistical analysis

The total pectin, DE, metal-ions, and DSC measurement were carried out in triplicate. Data were analysed by an ANOVA and Duncan's multiple-range test (mean comparison) by using the Statistical Analysis System program (SAS, 1985).

RESULTS AND DISCUSSION

The relationships between the changes of DE values and total pectins in the AIS and the textural changes of snap bean pods after different cooking treatments

Because the pectin is the major polysaccharide (approximately 40%, on dry basis) in the AIS of snap bean pods (Chang & Chang, 1992*b*) and the textural changes of vegetables were mainly caused by the changes in the linkages between pectin molecules, which has been recognised in many reports (Manabe, 1980; Loh *et al.*, 1982; McFeeters *et al.*, 1985; Chang *et al.*, 1993), we first investigated the changes of the DE values and total pectins in the AIS, and the textural changes of snap bean pods after different cooking treatments. In Fig. 1, the total pectins in the AIS of snap bean pods after different cooking treatments were not significantly different ($P > 0.05$). However, the DE values of the pectins in the AIS of the bean pods after precooking, and precooking followed by cooking, were significantly lower ($P < 0.05$) than that of the bean pods after direct cooking. The tendency of the difference in DE values was inversely related to the difference in tissue firmness. These results revealed that the firming effect of precooking for snap bean pods was in accordance with the action of pectinesterase on the pectic substances, which resulted in de-esterification of pectin molecules and the subsequent formation of calcium bridges between the adjacent pectin molecules.

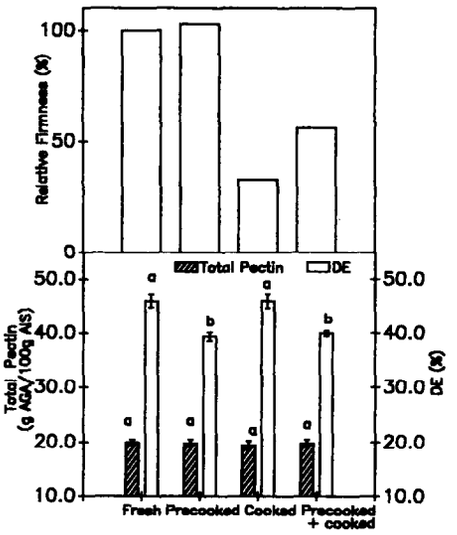

Fig. 1. Changes in firmness, total pectin, and degree of esterification (DE) of pectins in the alcohol-insoluble solids of snap bean pods after 15 min cooking in boiling distilled water, with or without precooking (70°C, 20 min). Columns under identical letter within the same category are not significantly different at $P > 0.05$.

The relationships between the changes of the contents of calcium and magnesium ions in the AIS and the textural changes of snap bean pods after different cooking treatments

The addition of divalent metal ions, such as calcium and magnesium, often aids the increase of tissue firmness of vegetables during processing. This results from the formation of metal ion-bridges between the free carboxyl groups of adjacent pectin molecules. The contents of calcium and magnesium ions in the AIS of snap bean pods are shown together with the relative firmness of the tissues after different cooking treatments in Fig. 2.

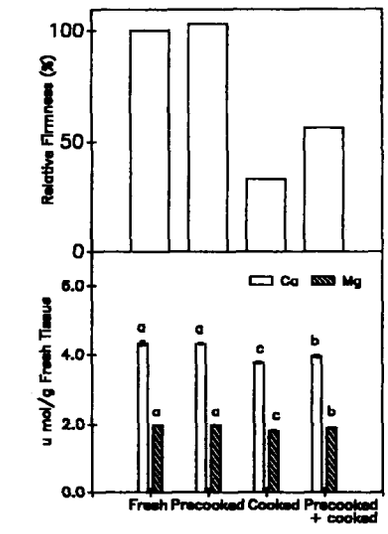

Fig. 2. Changes in firmness and metal ions in the alcohol-insoluble solids of snap bean pods after 15 min cooking in boiling distilled water, with or without precooking (70°C, 20 min). Columns under identical letter within the same metal category are not significantly different at $P > 0.05$.

Table 1. The correlation coefficients between firmness and the amounts of non-freezing and freezing water, and various metal ions in the alcohol-insoluble solids of snap bean pods before and after 15 min cooking in boiling distilled water, with or without precooking (70°C, 20 min)

	Ca	Mg	Non-freezing Water	Freezing Water	Firmness
Ca	1.000	0.947**	0.804**	−0.804**	0.980**
Mg		1.000	0.718**	−0.718**	0.956**
Non-freezing water			1.000	−1.000**	0.681*
Freezing water				1.000	−0.681*
Firmness					1.000

*$P < 0.05$; ** $P < 0.01$.

In Fig. 2, it is observed that the contents of calcium and magnesium ions in the AIS of precooked bean pods are significantly higher ($P < 0.05$) than those in the AIS of the bean pods with direct cooking. The trend of the differences between these metal-ion contents was the same as that of the relative tissue firmness of snap bean pods after different cooking treatments. Table 1 shows that the correlation coefficients between the firmness and the contents of calcium and magnesium ions in the AIS of snap bean pods after different cooking treatments were very high and significant ($P < 0.01$). This again shows the firmer texture of snap bean pods after precooking resulted from the formation of calcium or magnesium bridges between the free carboxyl groups of adjacent pectin molecules.

The relationships between the changes of the amounts of non-freezing water in the AIS and the textural changes of snap bean pods after different cooking treatments

It was observed from the above experiments that the linkages between pectin molecules in the tissues of snap bean pods after different cooking treatments might be changed. This would affect the state of the water in the pectin polymer and result in the changes of the amounts of free water (freezing water) and non-freezing water. The amounts of freezing and non-freezing water in the AIS of snap bean pods after different cooking treatments were determined by using DSC, which was modified from the method of Sawayama & Kawabata (1991), and results are shown in Fig. 3 and Table 2.

Table 2. The enthalpy changes and amounts of non-freezing and freezing water of the alcohol-insoluble solids of snap bean pods before and after 15 min cooking in boiling distilled water, with or without precooking (70°C, 20 min)

Cooking Treatment	Enthalpy Change (J/g)	Non-freezing Water (%)	Freezing[1] Water (%)
Fresh	211.3b[2]	35.8a	64.2b
Precooked	220.4b	33.1a	66.9b
Cooked	236.7a	28.1b	71.9a
Precooked + Cooked	231.9ab	29.6ab	70.4ab

[1]The amount of freezing water was calculated by dividing the enthalpy change of the sample by that of pure water, and this calculated amount was subtracted from the total water content in the sample to give the amount of non-freezing water. [2]Means with identical letter in each column are not significantly different at $P > 0.05$.

In Fig. 3, the DSC thermograms of distilled water and the citrus pectin with 80% moisture content showed an endothermic peak at a temperature near 0°C, which resulted from the thawing of ice. From the area of the endothermic peak, the enthalpy change of the thawing ice could be obtained, and then the amounts of freezing and non-freezing water could be calculated from this enthalpy change. Table 2 shows that the amounts of non-freezing water in the AIS of fresh and precooked bean pods were larger than those in the AIS of the bean pods after direct cooking. The amounts of non-freezing water had a significantly positive correlation to the tissue firmness ($P < 0.05$) (shown in Table 1).

From these results, it can be further confirmed that the linkages between the pectin molecules in the tissues of snap bean pods after precooking were really changed. This change was caused by the formation of calcium or magnesium bridges between the free carboxyl groups of pectin molecules by the action of pectinesterase and

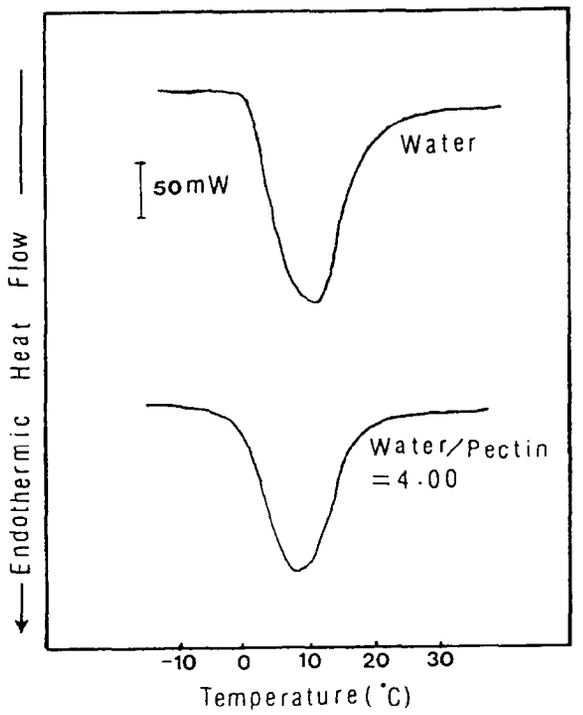

Fig. 3. The DSC thermograms of distilled water and citrus pectin. * Pectin was obtained from Sigma Chem. Co., USA.

resulted in increases in the amount of water clathrated or bound within the pectin polymer (i.e. non-freezing water) and in the firm tissue components of snap bean pods.

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