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Thermal Transitions of Melon Seed Proteins

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ABSTRACT

The heat of denaturation of the protein in melon seed products was studied by differential scanning calorimetry. At a heating rate of 10 °C/min, the protein in the raw seed, oil meal and flour showed endothermic transitions with the maximum heat input occurring at 160, 89 and 93 °C, respectively. Comparison with isolated melon seed protein fractions indicated that these transitions corresponded to the denaturation of the reserve globulin. The enthalpies of denaturation were 11·3 ± 1·3, 10·5 ± 0·8 and 12·1 ± 0·8 J/g for the raw seed, oil meal and flour, respectively. The water-soluble protein and the glutelin fractions did not show any observable transitions. Milling and defatting processes did not appear to denature the glubulin. However, pH and salts in the suspending medium affected its thermal stability. The heat-induced aggregation of melon seed protein was also studied. Aggregation of the water-soluble protein began at 60 °C and the globulin at 81 °C. The hot paste viscosity of a 10% w/v dispersion of melon seed flour reached a maximum of 80 Brabender units at 83 °C.

INTRODUCTION

Melons (*Colocynthis citrullus* Linn.) are grown in Nigeria primarily for their seeds which are rich in protein and oil (Oyenuga & Fetuga, 1975;

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Oyolu, 1977; Onuora & King, 1983). The oil meal prepared from the seed kernel is an important condiment in traditional soups and stews, in which it performs thickening and emulsifying functions.

It is proposed to adapt modern oil seed technology to process melon into oil- and protein-rich flour. The latter could be used in traditional or novel food products. The application of heat is a common feature of the techniques for defatting oil seeds and for the preparation of traditional melon seed products. Such heat treatments affect the physico-chemical properties of the resulting protein and hence the potential food application of the seeds. Therefore, the conditions under which food proteins undergo thermal changes such as denaturation, aggregation and coagulation, need to be investigated.

MATERIALS AND METHODS

Source of materials

Whole sun-dried melon seeds corresponding to Oyolu's type 5 (Oyolu, 1977) were obtained from a wholesale dealer in Enugu, Nigeria, air freighted to the United Kingdom and stored at 2 °C until required.

A melon seed oil meal (MSM) was prepared by milling the kernels in a Glen Creston S80 laboratory sample mill (Glen Creston, Stanmore, Middlesex, Great Britain) to an oily paste.

Unheated solvent defatted melon seed flour (MSF), total salt extractable protein (TSSP), water-soluble protein (WSP), globulin (GLOB) and glutelin (GLUT) were prepared as previously reported (King & Onuora, 1984).

Differential scanning calorimetry

Measurements were made using a Perkin-Elmer Differential Calorimeter 2. Samples of melon seed flours, meals and isolates were prepared as slurries using a tenfold excess of water, pH 6·4, and a high speed homogenizer to ensure dispersion of the protein. Measurements were also made on the whole kernels in the absence of water. Each sample was hermetically sealed and scanned from 27 to 127 °C at a heating rate of 10 °C/min. The instrumental sensitivity was 8·4 mJ/s.

When thermal transitions were evident, temperatures corresponding to

the departure and the return to the transition baseline and the maximum amplitude were recorded, together with the transition area and width. The enthalpy of transition was then calculated.

Aggregation

Turbidity measurements at 420 nm were used to follow the aggregation of the melon seed protein using the procedure of Hermansson (1978).

Hot Paste Viscosity

A Brabender viscograph (Brabender, Duisburg, West Germany) was used to measure the hot paste viscosity of a suspension of 40 g of melon seed flour in 450 ml of distilled water. The suspension was heated in the viscograph at a constant rate of 1·5 °C/min from 25 to 95 °C. The temperature was maintained at 95 °C for 5 min, after which the paste was cooled to 25 °C at 1·5 °C/min.

RESULTS AND DISCUSSION

Heat denaturation

The denaturation of melon seed protein and protein extracts from melon seed was studied by differential scanning calorimetry (DSC). Only one distinct endothermic peak was observed in each of the thermograms (Table 1). The same heating rate was used for all samples. Peak widths and the temperature of maximum heat input (T_{max}) differ for the various protein products. The highest value of T_{max} recorded was 160 °C for the melon seed kernels and the lowest 83 °C for the total salt-extractable protein fraction. No peak was observed for the glutelin fraction (GLUT). This is probably the result of the protein being denatured as the melon seed flour was extracted with 70 % aqueous ethanol prior to the extraction of this fraction. Also, the water-soluble fractions (WSP) did not melt in the temperature range used or, if they did, the process was not co-operative and therefore invisible to DSC.

The difference between the T_{max} values for the globulin fraction and for the total salt-extractable protein is probably due to the latter being a mixture of proteins corresponding to the globulin extract (GLOB) and the water-soluble protein (King & Onuora, 1984). The similarity between

TABLE 1
Characteristics of Thermal Transitions of Melon Seed Proteins Observed Using Differential Scanning Calorimetry

Sample[a]	*Temperature of maximum heat input* (°C)[b]	*Peak width* (°C)[b]	*Enthalpy of denaturation* (*J/g protein*)
Raw melon seed (4)	160 ± 2	9 + 1	11·3 ± 1·3
Melon seed oil meal (4)	89 ± 1	11 ± 1	10·5 ± 1·3
Melon seed flour, MSF (4)	93 ± 1	8 ± 1	11·3 ± 0·8
Total salt-extractable protein, TSSP (6)	83 ± 1	15 ± 1	6·7 ± 0·8
Globulin, GLOB (6)	90 ± 1	13 ± 1	12·1 ± 0·8
Glutelin, GLUT	[c]	—	—
Water-soluble protein, WSP	[c]	—	—

[a] Number of replicate determinations in parentheses.
[b] The variation shown is the standard deviation.
[c] No detectable response.

the T_{max} values for the isolated globulin (GLOB), the melon seed flour and the melon seed meal suggests that the observed transitions in the last two mentioned are attributable to the presence in them of globulin. The main difference between the melon seed flour and the melon seed meal is the absence of fat in the former. This therefore suggests that the presence of the fat lowers the heat stability of the meal. The enthalpies of denaturation (Table 1) are a measure of the nativeness of the protein in the samples. The lack of a significant difference between the enthalpies of denaturation for the melon seed flour, melon seed meal, globulin and the protein in the raw seed flour, melon seed meal, globulin and the protein in the raw seed shows that the milling and defatting processes did not significantly change the structure of the melon seed reserve globulin. The significantly lower value for the total salt-extractable protein is not unexpected as it contains non-DSC observable protein (WSP). The enthalpy of denaturation of the melon seed globulin (12·1 J/g) is less than the value reported for cowpea water-soluble protein (13·9 J/g) (Sefa-Dedeh & Stanley, 1979) and actin (14·5 J/g) (Wright *et al.*, 1977).

Effects of pH and salts in the suspending medium

The effects of pH and the presence of salt in the suspending medium on the denaturation of melon seed globulin were studied (Table 2). In water,

TABLE 2
Effects of pH and Salts on the Thermal Transitions of Melon Seed Globulin

Dispersing medium	*Temperature of maximum heat input (°C)*[a]	*Peak width (°C)*[a]	*Enthalpy of denaturation (J/g protein)*[a]
Water, pH 4	—[b]	—	—
Water, pH 10	79 ± 1	19 ± 2	13·4 ± 2·9
Water, pH 10	102 ± 1	11 ± 2	—
0·2M sodium chloride, pH 7	96 ± 1	10 ± 2	12·1 ± 2·5
1M sodium chloride, pH 7	98 ± 2	10 ± 1	0·63 ± 0·04
0·2M calcium chloride, pH 7	93 ± 1	12 ± 2	10·5 ± 2·9

[a] Mean of four replicates, variation shown is the standard deviation.
[b] No detectable transition.

at pH 4, the globulin showed no detectable response. At pH 10 it exhibited two transitions, the major one with T_{max} at 79 °C and a minor one at 102 °C. The lack of transition at pH 4, the pH of minimum solubility of melon protein (Onuora & King, 1983) indicates that the protein is denatured by acid precipitation. Examination of the melon seed globulin using electrophoresis and gel chromatography have shown it to be heterogeneous (King & Onuora, 1984). The more complex response at pH 10 is probably due to changes in the structure of the component proteins as a result of the alkaline conditions. Wright *et al.* (1977) reported that myosin displays either one, two or three transitions depending on the pH and ionic strength of the suspending medium. However, these workers attributed these to three regions of the myosin with differing thermal stability. The increase in the denaturation temperature (T_{max}) in salt solutions suggests that salts stabilize the structure of the globulin. This is supported by the fact that the enthalpy of denaturation of the globulin in 0·2M sodium chloride and in 0·2M calcium chloride (Table 2) are not significantly different from that of the globulin in water and the whole seed. In 1M sodium chloride T_{max} is higher (Table 2), implying a conferment of heat stability and the enthalpy of denaturation is very low, implying a very significant loss of native structure. Hermansson (1978) reported an increase in T_{max} for soy protein with salt concentration, whereas Quin *et al.* (1980) found that salts destabilize muscle protein so that they denature and coagulate at lower temperatures.

Aggregation studies

The heat-induced aggregation of soluble melon seed proteins was also investigated. Aggregation of the water-soluble protein in distilled water at pH 6·4 starts at 60 °C, coagulation and precipitation occurring at 85 °C (Fig. 1). Protein aggregation usually occurs after denaturation (Joly, 1965). In order to compare the aggregation behaviour of the water-soluble protein, globulin and total salt extractable protein, it was

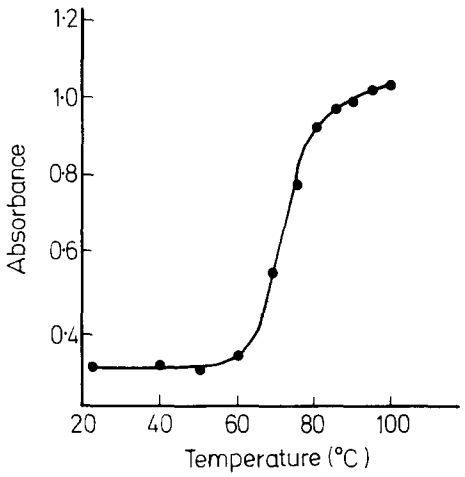

Fig. 1. Heat-induced aggregation of water-soluble melon seed protein, from turbidity measurements at 420 nm.

necessary to dissolve them in 1 M sodium chloride solution. The lower temperature for the onset of aggregation in the total salt-extractable protein solution compared with the globulin (Fig. 2) accords with the lower denaturation temperature of total salt-extractable protein relative to globulin and may also be attributed to the WSP component in the total salt-extractable protein.

Hot paste viscosity

On heating a 10 % w/v dispersion of melon seed flour in water the viscosity was found to increase after 81 °C, reaching a maximum of 80 Brabender units at 83 °C. It is assumed that this is due principally to the thermally

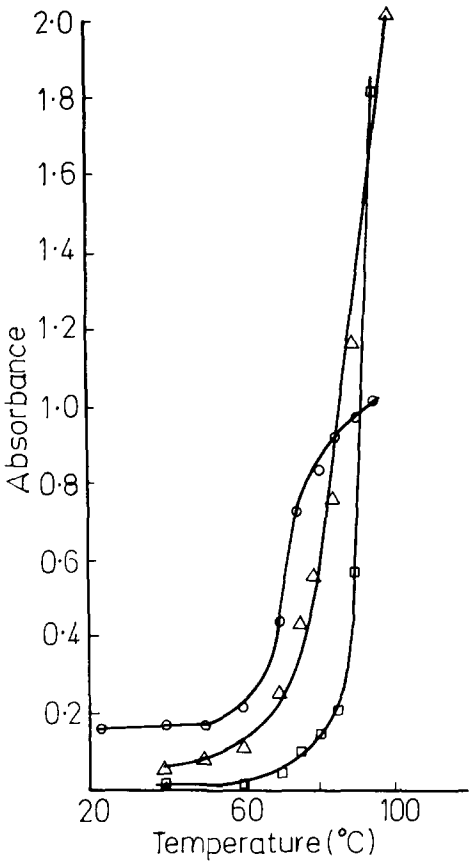

Fig. 2. Heat-induced aggregation of melon seed proteins in 1M sodium chloride from turbidity measurements at 420 nm; ○, water-soluble protein; △, total salt-extractable protein; □, globulin.

induced aggregation and coagulation of the melon seed protein as there is little starch in melon seed.

CONCLUSIONS

Differential scanning calorimetry heat aggregation studies and hot paste viscosity data of aqueous dispersions of melon seed globulins show that, between 80 and 90 °C these major proteins denature and aggregate.

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