

$$\text{Reducing sugar (\%)} = \frac{\text{mg of inverted sugar} * \text{Dilution titer}}{\text{titer} * \text{weight of the sample (g)} * 1000} \times 100$$

$$\text{Dilution factor} = \frac{\text{Sample volume}}{250}$$

For invert sugar, the following calculations and standardization of Fehling solution were carried out using about 9.5 g of AR-grade sucrose dissolved in 100 ml of distilled water. After the sucrose was dissolved, 5 ml of HCl was added, and the mixture was allowed to remain for three days at room temperature in order to cause inversion. In order to calculate the Fehling solution factors, 25 ml of an inverted sugar solution was transferred into a 100ml volumetric flask, diluted to volume (1ml is equal to 2.5 mg of inverted sugar), and then transferred to a burette with an offset tip. The endpoint was identified by the complete discoloration of the methylene blue indicator, which was used to titrate against the Fehling solution. The mg of inverted sugar was calculated using the formula below:

$$\begin{aligned} \text{Factor for Fehling Solution (g of inverted sugar)} \\ = \frac{\text{Titer} * 2.5}{1000} \end{aligned}$$

Determination of pH Value

A digital pH meter (Model 744, Metrohm, Switzerland) was used to measure the pH of orange peel [12].

Characterization of Extracted Pectin

Determination of Yield

The extraction yields of pectin from three varieties of orange peels on a dry weight basis were determined as follows:

$$\text{Pectin yield (\%)} = \frac{\text{weight of dried pectin (g)}}{\text{weight of dried orange peel powder (g)}} \times 100$$

Determination of Viscosity

The resultant pectin was dissolved in distilled water at a concentration of 1.0 percent (weight per volume). A viscometer (Model HTD13145, Haitongda, China) was used to measure the mixture's viscosity at 100 revolutions per minute at about 22°C.

Determination of Equivalent Weight (EW)

The equivalent weight of pectin obtained from orange peels was determined [13]. This required 0.5

g of pectin sample with 5 ml of ethanol, then mixed against 0.1 N NaOH, one gram of sodium chloride, 100 milliliters of distilled water, and six drops of phenol red indicator. The titration point was designated by the color purple. The following equation was used to calculate the equivalent weight (EW).

$$\text{Equivalent weight (EW)} = \frac{\text{weight of pectin sample (g)}}{\text{mL of NaOH} * \text{N of NaOH}} \times 100$$

Determination of Methoxyl, Degree of Esterification, and Anhydrouronic Acid

The degree of esterification (DE) is defined as the ratio of the esterified galacturonic acid group to the galacturonic acid group present. The degree of esterification was determined through titration, connecting the methoxyl content (MeO) with the equivalent weight method [13]. First, 0.50 g of orange peel pectin was dissolved in a 1:20 v/v solution of ethanol and water. Next, the mixture was titrated with 0.1 N sodium hydroxide (V1, ml) until the indicator changed, after which five drops of the phenol red indicator were added. The sample, heated briefly, was vigorously stirred after adding 25 ml of 0.25 N NaOH. Five drops of phenol red and 25 ml of 0.25 N HCl were subsequently added. After that, 0.1 N NaOH (V2, ml) was added to the mixture in titrations until the endpoint color changed from yellow to pale pink. The following equation was then used to calculate the MeO and the DE.

$$\text{MeO (\%)} = \frac{V2(\text{ml}) * 0.1\text{N} * 31}{\text{weight of pectin sample (mg)}} \times 100$$

$$\text{DE (\%)} = \frac{V2(\text{ml})}{V2(\text{ml}) + V1(\text{ml})} \times 100$$

The anhydrouronic acid (AUA) content of pectin was also calculated as follows:

$$\text{AUA (\%)} = \frac{0.1(\text{N}) * (V1(\text{ml}) + V2(\text{ml})) * 176}{\text{weight of sample (mg)}} \times 100$$

Where V_1 and V_2 are the volumes used for the first and second titrations, respectively, 31 is the molecular weight of methoxyl and 176 is the molecular weight of anhydrouronic acid expressed in mg/meq.

Determination of Acetyl Content

The acetyl content was measured [13] using 0.5 g of pectin mixed with 25 ml of 0.1 N NaOH, followed by rapid agitation to ensure thorough mixing, then diluted with 50 ml of diluent after being left for 1 h. The 20 ml of liquor was put into a steam distillation device

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