# **The Effect of Pectinase Enzyme Pre-Treatment on the Physiological Characteristic and Nutritional Properties of Raisin Spread**

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**ABSTRACT**

Raisins, or “Kishmish” as they are sometimes known, are dried grapes consumed worldwide. They are a great addition to baking, cooking, and brewing and provide various health benefits. Raisins are surprisingly full of nutrients like potassium, magnesium, and iron and free from saturated fat and cholesterol, they are rich and concentrated sources of energy, vitamins, electrolytes, and minerals. Raisins possess remarkable antioxidant and antibacterial qualities that some specific polyphenols, such as quercetin, procyanidins, and catechin, are accountable for raisins’ antioxidant and antibacterial properties. Eating foods high in antioxidants, mainly phenolic compounds like flavonoids is essential to managing and preventing diabetes. by preventing the development of insulin resistance and protecting cells by reducing oxidative stress damage. Additionally, [research](https://pubmed.ncbi.nlm.nih.gov/23789931/) indicates that raisins may benefit cardiovascular health.

The present study addresses preparing and formulating a new product, **Raisin spread**. The aim of the study is to examine the effect of the pectinase enzyme (in three different levels of 0, 500, and 1000 ppm) and concentration temperature (65, 75, and 85°C) on pH, acidity, Brix, colour, and sensory parameters. The result indicated that by increasing the enzyme, pH, and colour parameters were increased but the identity and Brix were decreased. Also, the results showed that by increasing the concentration temperature from 65°C to 85°C, colour parameters were decreased, and acidity increased. On the other hand, sensory analyses showed the sample with 500 ppm of pectinase enzyme me and a concentration temperature of 75°C was the best treatment in this study so we use it for further analyses to determine its rheological characteristics like apparent viscosity and its nutritional properties such as heavy metals along with its physicochemical properties.

**Keywords**: Raisin spread, pectinase enzyme, formulation, physiological nutritional properties

**INTRODUCTION**

Raisins are dried grapes most widely recognized dried natural product in almost all aspects of the world. Be that as it may, all grapes are not appropriate for making raisins. (*Maynard et al. 2003*). The sweet grapes are just chosen for drying. Raisins have high feeding characteristics. Raisins are regarded for their uncommon food esteem and are plentiful in starches, minerals, and nutrients. Furthermore, they can be utilized in nearly everyone's food diet (*Zuritz et al, 2005*). Raisins are a rich source of probiotics that aid in the prevention of cancers and chronic diseases.

A prebiotic has been recently defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon’’ (*Gibson GR and Roberfroid MB, 1995*). Sun-dried raisins contain 5.7g fructans per 100g of fruit (*Camire ME and Dougherty, 2003*) higher than all commonly consumed fruits (*Muir JG et al, 2007*)

Several studies address raisin's therapeutic properties (*Thea, 200The present study introduces new raisins of raisin called raisin produced*. Based on previous studies and the nutritional value of raisins *Zuritz et al, 2005*) and particularly a growing need to include much more healthy food made from fresh healthy raw material in people’s diet, raisin spread was made from sun-dried seedless raisins.

The effect of pectinase enzyme and concentration temperature on the product’s final Properties was evaluated, at three different levels of 0, 500, and 1000 ppm and 65, 75, and 85°C respectively.

**MATERIALS AND METHODS**

***Material***

The sun-dried seedless raisin and sugar were obtained from the local market in Iran. Pectinase enzyme was obtained from Shahd-e-Iran Factory (Iran), and citric acid was obtained from Merck (Germany).

***Methods***

Raisins were cleaned, washed, and kept in polyethylene bags at room temperature (28 °C) for further processes. Raisins were dipped in boiling water for 15 minutes, crushed by a 60-rpm mixer, and filtered by a cloth mesh. For pectin digestion, the enzyme was added in three different levels and for better enzyme functioning, the mixture was kept at 45 °C for 2 hours. And it was filtered again.

Then sugar was added, and concentration was continued at three temperatures (65, 75, and 85 °C) for 30 minutes. Finally dissolved citric acid in water was added to the concentrate and the mixture was filled in Glass jars and stored at room temperature for further analysis.

***Physic-chemical analysis***

***PH***

PH was measured by a Metrohm 691 pH meter. A 15% solution of the raisin spread was prepared with twice distilled water; mixed for 15 minutes with a magnetic mixer, then rest for 10 minutes at room temperature. And then pH was measured (*Arunepanlop et al, 1996*).

***Acidity***

Acidity was measured according to ISIRI No.214 (*Institute of Standards and Industrial Research of Iran*).10 gr of the raisin spread was diluted in distilled water to the volume of 100 ccs, the mixture was filtered and 25 cc was titrated with NaOH 0.1N and phenolphthalein indicator until a light pink color appeared. Acidity was measured by:

$$Acidity=\frac{0.00064×100×a}{10×s} ×100$$

Where: ***a***, is the volume of NaOH, and ***s***, is raisin the spread volume (here 25cc).

***Brix***

Brix was measured by a hand refractometer (*AOAC, 2005*).

***Colouring Components Analysis***

This was measured by describing L\*, a\*, and b\* indexes. L\* value shows the lightness of the product and is ranged from 0 (pure black) to 100 (pure white). A\* value shows red and green and ranged from -120 (pure green) to +120 (pure red), while b\* value e is blue and yellow and ranges from -120 (pure blue) to +120 (pure yellow). 26 gr of raisin spread was placed in a glass plate to form a thin layer. Images were taken by a HP Scanjet G3010. The images were measured by Image J (*Sun, 2008*).

***Sensory Evaluation***

This evaluation was carried out based on a five-point hedonic scale, using 10 panellists to evaluate the sample by grading the sensory properties such as colour, consistency, firmness, adhesiveness, spreadability, and overall acceptability. Evaluation factors were from poor (1) to excellent (5) (*Harry, 2010*)

***Nutrients***

The best treatment was evaluated on the nutritional value, Iron and Calcium. Table 1 briefly shows these nutrients and the standard method used.

|  |
| --- |
| **Table 1: Standards methods for evaluating nutritional value.** |
| **Nutritional value** | **Weight** |
| National Standard 9266 | Iron (mg/kg) |
| AOAC 985-35 | Calcium(mg/kg) |
|  | Total carbohydrates based on  |
| National Standard 2303 | Glucose amount(%weight) |
|  | Glucose, Fructose |
| National Standard 92 | Saccharose and reducing |
|  | Sugar(%weight) |
| National Standard 3105 | Fibre |
| AACC 30-10 | Fat  |
| AACC 46-10 | Protein  |
| AACC 08-01 | Ash  |

***Viscosity***

A viscosity test was carried out on the best treatment using a rotary Bohlin viscometer at 25°C with thermostat circulating water under precise temperature control (±0.1) (*Dabir et al, 2006*).

Shear stress was increased from 14 to 400 s-1, and apparent viscosity was measured on the shear stress 100 s-1. The rheological properties were calculated based on Power-Law and Herschel- Bulkley formulas (Anandha Rao, 2007).

***Statistical analysis***

The samples were prepared in three replicates and the results were analyzed by Mstat–C, 1.42 version, using a 2 x 2 elements factorial design; adding pectinase enzyme in three different levels of 0, 500 and1000 ppm and three concentration temperatures 65,75 and 85°C. A multiple comparison procedure of the treatment means was performed by Duncan’s New Multiple Range Test. The significance of the differences was defined as P<0.05. All diagrams were drawn in Excel.

**RESULT AND DISCUSSION**

***PH***

With increasing levels of pectinase enzyme in the raisin spread formulation, PH levels in each sample, relative to the control sample (sample without pectinase enzyme at the same concentration temperature) were significantly (P<0.05) increased. On the other hand, results show that with increasing the concentration temperature, PH levels in each sample were significantly (P<0.05) increased (Figure 1).

***Acidity***

By increasing enzyme levels in raisin spread, acidity decreased, this decrease was not meaningful. Results showed that increasing concentrating temperature would cause acidity to increase (figure 2).

***Brix***

Results show that with increasing enzyme levels in raisin spread, Brix would decrease, and with increasing concentrating temperature Brix would increase relative to the control sample, meaningfully.

The highest Brix belongs to the sample with 0 ppm enzyme and a concentration temperature of 85°C, and the lowest was for the sample containing 1000 ppm at 65°C (figure 3).

***Lab***

Increasing enzyme caused a meaningful increase in L\*, a\*, and b\* values, relative to the control sample. But Concentration temperature caused a meaningful decrease in all these three factors (Figures 4, 5, and 6).

***Sensory evaluation***

Adding enzyme up to 500 ppm caused an ascending trend in scoring the samples, but further addition of enzyme or less than 500 ppm, reduced the sample’s score in sensory evaluation, which might be an outcome of pale and un-natural sample colour, negative change in samples’ texture, low consistency, unfavourable firmness, adhesiveness, and spreadability caused by extra enzyme addition, which in return reduced the total acceptability of the product.

On the other hand, increasing the temperature to more than 75 °C caused a reduced score for samples because of a dark color, un-favorable caramelization taste, low consistency, poor texture, and undesirable firmness, adhesiveness, and finally reducing the overall acceptability.

Based on the gained results, the best treatment was the one having 500 ppm enzyme and a concentration temperature of 75 °C. So, its rheological properties, viscosity, and nutrients were studied.

***Viscosity***

Tables 2 and 3 show the rheological properties based on Power-Law and Herschel-Bulkley models.

**Table 2: Viscosity of raisin spread by Power Law model.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Samples** | **R 2** | **n(-)** | **K (pa. sn)** |
| 1st  | 0.993 | 0.58 | 32.98 |
| 2nd | 0.989 | 0.58 | 33.47 |
| 3rd | 0.989 | 0.64 | 36.74 |
| Mean | 0.999±0.002 | 0.60±0.035 | 34.40±2.04 |

**Table 3: Viscosity of raisin spread by Herschel- Bulkley model.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Samples** | **R2** | **N (-)** | **K (pa. sn)** | **I0** |
| 1st | 0.994 | 0.68 | 11.03 | 98.38 |
| 2nd | 0.997 | 0.80 | 7.12 | 141.68 |
| 3rd | 0.998 | 0.80 | 8.34 | 162.20 |
| Mean | 0.995±0.002 | 0.76±0.07 | 8.36±2.31 | 134.09±32.58 |

**Table 4: Apparent viscosity of raisin spread.**

|  |  |
| --- | --- |
| **Sample** | **Apparent Viscosity (mpa.s)** |
| 1st | 4767.04 |
| 2nd | 4838.887 |
| 3rd | 7000.633 |
| Mean | 5535.20±1269.62 |

***Nutrients***

 Results of the physicochemical analysis for the best sample of the raisin spread, including total carbohydrate, Glucose, Fructose, Saccharose, reducing sugars, Raw Fiber, Total ash, Protein, Fat, Iron, and Calcium are presented in table 5.

**Table 5: Nutrition value of raisin spread.**

|  |  |
| --- | --- |
| **Nutrients** |  **Weight %** |
| Total carbohydrate  |  64.85 |
| Glucose  |  36.84 |
| Fructose |  28.81 |
| Saccharose |  4.2 |
| Reducing sugar |  64.85 |
| Fiber |  0.13 |
| Total Ash |  0.58 |
| Protein (6.250 |  1.15 |
| Fat |  0.75 |
| Iron |  8.20 |
| Calcium  |  234.70 |

**CONCLUSION**

This study evaluated the effects of adding pectinase enzyme (0, 500, and 1000 ppm) and concentration temperature (65, 75, and 85 °C) on raisin spread. Results indicated that adding enzyme from 0 ppm to 1000 ppm caused an increase in pH, Lab but on the other hand reduced the amounts of acidity and brix.

Also, it was observed that temperature played an important role in the physiological and nutritional properties of raisin spread since an increase in temperature from 65 to 85 °C caused an increase in acidity and brix but decreased pH and Lab.

On the other hand, evaluation of independent effects of temperature and enzyme amount on raisin spread indicated that the scoring trend was ascending to 500 ppm enzyme at 75 °C, and additional enzyme and heat made that a descending trend. Based on all results, the best treatment was considered as a sample with 500 ppm enzyme and a concentration temperature of 75 °C, so further tests were recommended to be carried out on this sample to clarify its properties better.

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