Lhamo et al. 2022 Bhutanese Journal of Agriculture 5(1), 183-195
https://doi.org/10.55925/btagr.22.5115

Study on Clarification of Apple Juice using Enzymes

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ABSTRACT

One of the key challenges in apple juice processing is obtaining a good juice recovery and attaining a juice with good clarity. The presence of pectin and starch components inhibits the juice extraction process and leads to the formation of a cloudy haze which is undesirable in apple juice. For these purposes, maceration enzymes such as pectinase, amylase, cellulose, and hemicellulase are added both before and or after juice extraction to enhance juice recovery percentage and clarify the juice. Process parameters such as type of enzymes used, stage of enzyme addition, incubation time and temperature influence the efficiency of enzymes. The optimisation of these parameters is critically important in apple juice processing for better juice recovery and attaining the desired juice clarity. In this study, juice recovery percentage was compared amongst the control and three treatments- addition of enzyme pectinase at 0.02%, amylase at 0.02% and a combination of both at 0.01%. For the optimisation of process parameters, the type of enzymes used, stage of enzyme addition, incubation temperature, and time were studied as independent variables by comparing with the transmittance value as a dependent variable. Amylase at 0.02%, pectinase at 0.02%, and a combination of 0.01% each of both enzymes were used. The enzyme was added to the pomace and the juice. The treatments were incubated at 20^0C and 40^0C. Measurements were done after every 1, 2, 4, and 24 hours of incubation. The juice recovery percentage was not significantly different in the 3 treatments and control where the enzyme was added to the crushed apple. For clarification of apple juice, it is recommended to add the combination of 0.01% each of amylase and pectinase directly to the pomace before juice extraction and incubating the juice obtained at 40^0C for 24 hours.

Keywords: *Enzymes; Apple juice; Clarification; Juice recovery; Process parameter*

1. Introduction

Apple is one of the major cash crops grown in the country for export to Bangladesh and India. Apple is predominantly grown in Paro, Thimphu, Haa, Chhukha and Bumthang Dzongkhags. The main apple varieties grown in the country are Royal Delicious, Red Delicious and Golden Delicious (Choden & Shanawaz, 2015). Over the past 5 years from 2015 to 2019, an average of 5589 metric tonnes (t) of apples were produced in the country (RSD, 2020). Besides the export, two main agro-processing companies in the Country-Bhutan Agro Industries Limited

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in Thimphu and the Fruit Processing Enterprise in Bumthang utilize local apples as raw materials for processing products such as apple juice, beverages and jams.

In 2016, the total apple production was reported to be 6587 Mt (DoA, 2016) and about 8% of the total production volume amounting to 450 Mt was procured by Bhutan Agro Industries Ltd. (DAMC, 2017) for apple juice processing. Ready to serve apple juice is processed from fresh apples by crushing the washed and sorted raw apples and subsequent pressing the crushed apples by a frame filter press. The quality of apples used for juice processing determines the end quality of the juice, thus the genetic composition and other growth factors such as nutrition, climatic condition, maturity and storage of the apples affect the quality of the juice (Pollard & Timberlake, 1971).

Juice recovery from apple and clarity of the juice are two critical factors for ensuring the economic viability of the processing units and acceptance of the product in the market. The process of juice extraction involves rupturing of cells to release juice and pressing the ruptured mass of cells to extract the juice. Thus, apple juice contains the soluble constituent present in the apple which generally has 85% water, 10-12% carbohydrate, 1% pectin, 0.5 - 1% organic acid, 0.5% potassium, phenol, amino acid and flavouring in small amounts (Ryan, 1972). Pectic substance in apple is present in the cell wall and middle lamella. Pectin is a complex long-chain polysaccharide made of multiple units of (1,4)-a-D-polygalacturonic acid. Starch is present in the fruit as a reserve food and as the fruit ripens this starch gets converted into sugar. Apple juice obtained after crushing and pressing may contain up to 1% starch which presents a problem of cloudiness in the juice (Sorrivas, Genovese, & Lozano, 2006).

During the extraction process, the juice along with suspended particles comprising both water-soluble and insoluble materials leave the extractor. The presence of pectin in the juice inhibits the juice extraction process resulting in a low juice recovery rate (Root & Barrett, 1996). The presence of hydrophilic hydroxy group gives pectin its water-binding capacity, as a result, the juice binds to the pectin present in the pulp hindering the extraction process (Shiv, 2015). This also causes an increase in the viscosity of the juice and lubricates the crushed fruit pulp which results in slippage during pressing and as a result lowers the juice recovery rate (McLellan & Padilla-Zakour, 2004).

Consumer preference for clear apple juice (Kilara & Buren, 1989) makes it imperative to remove the cloudy haze present in apple juice. This cloudy haze is due to the presence of pectin and starch (Padma, Sravani, Mishra, Sneha, & Anuradha, 2017). The cloudy haze is formed

due to the reaction between starch with protein to form a positively charged protein-carbohydrate complex which acts as a positive core and attracts the negatively charged pectin. This particle is responsible for the cloudiness in apple juice (Yamasaki, Yasui, & Arima, 1964). The protein-carbohydrate complex surrounded by the protective pectin coat prevents aggregation of the particles resulting in a stable suspension thus hindering the process of sedimentation and subsequent filtration (Sorrivas et al., 2006).

The use of maceration enzymes such as pectinase breaks down the pectin structure and lowers its water-binding capacity, thus freeing up the juice bound to the pectin structure and improving the juice recovery rate. Pectinase enzymes digest the pectin by hydrolysing or de-esterifying pectin, as a result, pectin loses its water-binding capacity, flocculates and settles down as sediments. This process is known as depectinization (Kilara & Buren, 1989). Pectinase enzymes are naturally present in fruits and help convert the insoluble protopectin in unripe fruit to soluble pectin during ripening. However, naturally occurring pectinase enzymes are not sufficient enough to achieve adequate clarification in the juice, thus pectinases mostly from microbial sources are used for commercial juice processing (Patidar, Nighojkar, Kumar, & Nighojkar, 2018).

Amylase and pectinase are used individually and in combination to clarify apple juice to improve the juice recovery percentage. Studies have reported that when pectinase and amylase are used in combination, the synergistic effect has a more desirable effect on the clarity of the juice than when used individually (Padma et al., 2017). The use of a mix of enzymes during the clarification process can bring about both depectinization and destarching. For example, amylase is a starch degrading enzyme that works by hydrolysing the glyosidic linkage in long-chain starch breaking it into smaller units in a process known as destarching (Rana, Verma, Vaidya, & Dipta, 2017). Further, the synergistic effect entails the depectinisation reaction first followed by the prevention of possible agglomeration of the starch molecules with the protein-pectin complex formed as a result of depectinisation (Dey & Banerjee, 2014).

The enzyme is added at various stages of processing. For enhancing juice recovery percentage, the addition of enzymes takes place after the crushing process and before the pressing stage. For clarification purposes, enzymes are usually added to the juice after the pressing stage. To achieve both the purpose of enhanced juice recovery and clarification, some processors add enzymes at both stages (McLellan & Padilla-Zakour, 2004). Enzymic degradation of the haze forming components brought about by the macerating enzymes is responsible for the enhanced

juice recovery and clarification. The process of enzymic degradation is influenced by parameters such as the type of enzymes, dosage used, exposure time of the substrate to the enzyme, the temperature and the pH (Singh & Singh, 2015). For this study, only the type of enzymes used, the exposure time and temperature were observed. All the treatments were done at the original pH of the naturally extracted juice. This study attempted to replicate the industrial process used in the production of natural apple juice where alteration of pH is not commercially practised (Rai, Majumdar, Dasgupta, & De, 2004), but the process parameters such as types of enzymes used, duration of treatment and incubation temperature after the addition of enzymes were observed.

It has been reported by Kilara and Buren (1989) that at a commercial level, an enzyme dosage of 0.02% calculated on the volume of juice is used. Ezugwu et al. (2014) reported that at 40^0C the enzymes gave the most favourable result. To ensure the effectiveness of enzyme treatment in enhancing both the juice recovery and clarity of juice and also to have a cost-effective process, it is imperative to know the effect of the process parameters on their own and also in combination with other parameters for better optimization of apple juice processing.

Thus, the objectives of this study were:

a) to compare the juice recovery from apple pomace treated with 0.02% amylase, 0.02% pectinase and a combination of 0.01% amylase and pectinase before juice pressing, and
b) to compare the clarity of apple juice treated with amylase, pectinase or in a combination of amylase and pectinase and the effect of the process parameters such as incubation temperature, duration of treatment and stage of addition of enzyme on clarity of the apple juice.

2. Materials and Method

The experiment was conducted in the food analysis laboratory at the National Post Harvest Centre (NPHC), Paro. Apples stored in the cold store at 4 degrees Celsius were sorted, washed and the cores removed. For the extraction process, a fruit crusher and frame filter press were used. Processing equipment and utensils such as a fruit pulper, frame filter press, autoclave, stainless steel knife, plastic buckets, and stainless-steel stirrer were used for the process. Apples were first crushed in a pulping machine and the crushed apples were pressed using a hydraulic juice press. Enzyme dosage of 0.02% amylase, pectinase and 0.01% each of amylase and pectinase were added to 7-kg each of crushed apple before the pressing process. A control without any addition of enzyme was used. The weight of juice from triplicates of each treatment

and control was recorded and the juice recovery percentage was calculated using the following formula:

Juice recovery percentage (%) = [Volume of extracted juice (litre)/Weight of crushed apple used for juice extraction (kg)] ×100

The juice obtained from crushing the three treatments was taken as the three treatments when enzyme addition is done to the pomace before juice extraction. Enzyme dosage of 0.02% amylase, pectinase and 0.01% each of amylase and pectinase were added to 3.5 litres of apple juice extracted from the pomace without prior addition of enzymes. The juice obtained from control in the previous experiments was also taken as the control sample. 400 ml of juice from triplicates of all six treatments and control were transferred to glass jars. Two similar batches were prepared whereby each batch was placed in an electric dryer with the temperature set at 20^0C and 40^0C. Samples were drawn after 1, 2, 4 and 24-hour of incubation period and filtered for transmittance measurement. The juice was filtered using Whatman 5 filter paper. The sample drawn was immediately autoclaved at 100^0C for 5 minutes to de-activate the enzyme. The transmittance of the samples was measured at 660 nanometers using an advanced microprocessor UV-VIS Single Beam Spectrophotometer (LI-295). Distilled water was used as a blank to calibrate the spectrophotometer to give a transmittance value of 100% (Berutu, Fahrurrozi, & Meryandini, 2017).

Factorial ANOVA with main and combination effects was done to study if the enzyme type had a significant effect on the juice recovery. A two-way ANOVA and post hoc test was also done to study the main and interaction effect of enzyme type, temperature and time on the clarity of the extracted juice. The analysis was carried out using the Statistical Package for Social Science software (SPSS). *P*-values ≤ 0.05 were considered significant in all analyses.

3. Results and Discussion

3.1 Juice Recovery Percentage

The juice recovery percentage from the treatment treated with 0.02% of enzyme pectinase gave the highest juice recovery of 66.5% followed by the 0.02% amylase added sample. The 0.01% amylase and pectinase added sample which gave similar values of 62.5% and 62.8%, respectively. The control had the lowest value of 60.4%.

An ANOVA (Table 1) revealed that there is no significant difference ($F(3, 8) = 0.949$, $P = 0.461$). An increase of 6-7% in juice recovery was reported upon the addition of commercially available pectinase enzyme at a dosage of 0.01-0.05% (Oszmiański, Wojdyło, & Kolniak,

2009). However, in this study, the addition of enzymes did not significantly increase the juice recovery percentage. Though some studies report that the addition of enzymes increases the juice recovery percentage, the increase in juice recovery is also dependent on the commercial grade of enzymes used (Chang, Siddiq, Sinha, & Cash, 1995).

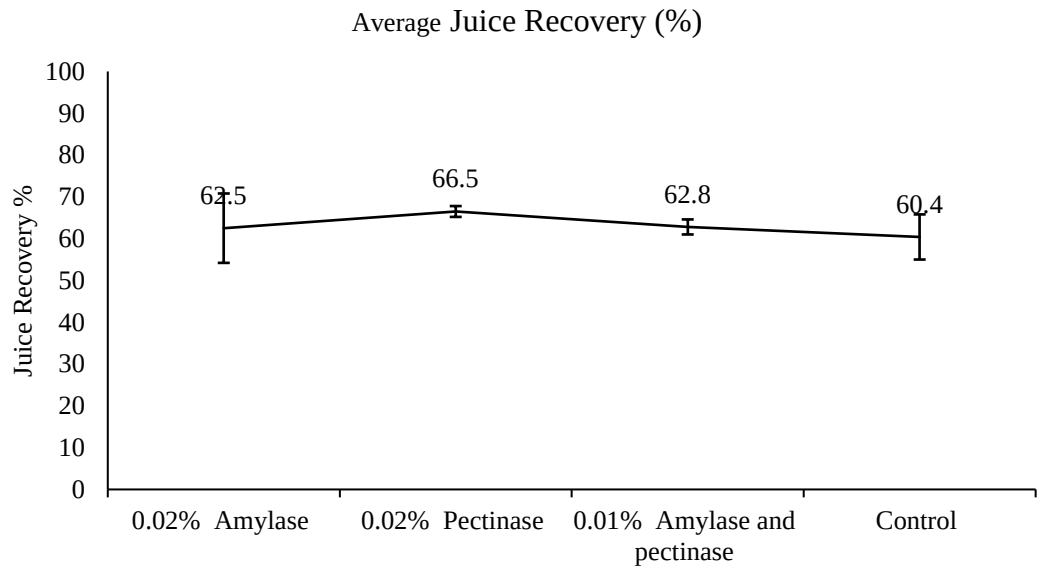

Figure 1. Average juice recovery percentage value

3.2 Transmittance Value

Transmittance value (expressed as percent transmittance) measured in spectrophotometer is the amount of light that passes through the sample (Garner, Crisosto, Wiley, & Crisosto, 2008), hence this value was used as a measure of clarity. A factorial Between-Subjects ANOVA with the experimental design 2X4X2X4 was performed to study the main and interaction effect of process parameters such as the stage of enzyme addition, treatments, incubation temperature and time on the transmittance value (Table 2). If the *P*- value of the parameters on their own and in combination is less than 0.01 then the parameters or the combination of parameters are reported to have a significant impact on the clarity of the juice. The partial eta squared is the value of the magnitude of this impact. The effect of the parameters both on their own and in combination are ranked in Table 1 from highest to lowest (1-7) in superscripts in the 5th column based on the partial eta squared values. The Treatment (F (2,112) = 151.788, $P = 0$, partial $\eta^2 = 0.730$) had the highest main effect on the transmittance value followed by incubation time (F (3,112) = 85.404, $P = 0$, partial $\eta^2 = 0.696$). The interaction effect of treatment and incubation time (F (6,112) = 37.757, $P = 0$, partial $\eta^2 = 0.637$) had the third-highest impact on the transmittance value. A similar finding was reported by Umsza-Guez et al. (2011) whereby the

clarity was significantly influenced by the enzyme and the incubation time**.** Though in total 3 main effects and six combined effects were detected, only the first 5 effects ranked according to the partial eta squared will be discussed.

Table 2. Test of between-subject effects

Source	df	F	Sig.	Partial Eta Squared
Corrected Model	55	23.316	.000	.920
Intercept	1	779.527	.000	.874
Stage of Enzyme addition	1	.019	.892	.000
Incubation Temperature	1	32.222	.000	.223[6]
Treatment	2	151.788	.000	.730[1]
Incubation time	3	85.404	.000	.696[2]
Before_after enzyme addition * Treatment	2	9.596	.000	.146[9]
Incubation temperature * Treatment	2	17.668	.000	.240[8]
Incubation temperature * Incubation time	3	13.025	.000	.259[7]
Treatment * Incubation time	6	32.757	.000	.637[3]
Before_after enzyme addition * Temperature * Treatment	2	1.790	.172	.031
Before and after enzyme * Temperature* Incubation Time	3	2.220	.090	.056
Before and after enzyme * Treatment* Incubation Time	6	8.567	.000	.315[5]
Temperature * Treatment * Incubation Time	6	15.701	.000	.457[4]
Error	112			
Total	168			
Corrected Total	167			

3.2.1 Main effect of treatment, incubation time and temperature on transmittance value

The main effect gives the stand-alone impact of a single process parameter without taking into consideration the effect of other parameters involved. The stand-alone effect of treatment type followed by incubation time had the highest main effect on the transmittance measurement. Incubation temperature also had an impact on the juice clarity. However, whether the enzyme is added to the pomace before juice extraction or to the juice after the extraction on its own without considering the other process, parameters did not seem to make much difference to the transmittance value.

Thus, a one-way ANOVA and post hoc Tuckey test was used to detect significant difference among the main effect of different treatment, incubation time and temperature. Both the ANOVA test for treatment ($F(3, 164) = 15.863$, $P = 0.000$) and incubation time $F(3, 164) = 17.115$, $P = 0.00$) revealed that there was a statistically significant difference in the mean transmittance values (Table 3 and Table 4 respectively). The post hoc test revealed that the

mix of enzyme added samples had significantly higher clarity followed by the pectinase added sample. The 0.02% amylase added sample had significantly lower clarity in comparison to the other treatment. A similar finding has been reported by (Padma et al., 2017) whereby the combination of pectinase and amylase enzyme was found to be more effective in achieving higher juice clarity than the individual enzyme on its own. In addition, studies conducted by Kothari, Kulkarini, and Baig (2013) reported that a combination of pectinase and amylase gave better transmittance value followed by the addition of pectinase on its own. They also reported that the amylase enzyme added samples gave the lowest transmittance value among the three.

The sample with the 24-hour incubation time also had a significantly higher clarity measurement than the samples incubated at 1, 2 and 4-hour. (Table 4). The ANOVA test for incubation temperature ($F(1, 166) = 6.689$, $P = 0.011$) revealed that there was a statistically significant difference in the mean transmittance values (Table 5) with the sample incubated at 40^0C having significantly higher clarity measurement than the one incubated at 20^0C.

Table 3 – One Way ANOVA & post hoc Tuckey test for different treatments

Treatment	Average Transmittance value with standard error
0.02% amylase	2.86 ± 0.31^{c}
0.02% pectinase	21.91 ± 3.05^{ab}
0.01% amylase and pectinase	26.39 ± 3.65^{a}
Control	12.14 ± 1.87^{b}

Means within a column with different superscripts differ significantly ($P\leq0.05$)

Table 4 – One Way ANOVA & post hoc Tuckey test for different incubation time

Incubation Time in hours	Average Transmittance value with standard error
1	9.17 ± 1.30^{bc}
2	18.37 ± 2.55^{b}
4	6.25 ± 1.09^{c}
24	31.62 ± 4.58^{a}

Means within a column with different superscripts differ significantly ($p\leq0.05$)

Table 5 – One Way ANOVA for different incubation temperature

Incubation Temperature in degree celsius	Average Transmittance value with standard error
20	12.37 ± 1.58^{b}
40	20.34 ± 2.64^{a}

Means within a column with different superscripts differ significantly ($P\leq0.05$)

3.2.2 Interaction effect of treatment, incubation time and temperature on transmittance value

As reported in the factorial ANOVA, the combination of treatment and incubation time had the highest interaction effect. The addition of a combination of enzymes incubated at 24 hours gave the highest clarity measurement and this effect is seen when the incubation temperature is 40^0C (Figures 2 & 3). The combined impact of treatment and time is more pronounced in the samples incubated at 40^0C than at 20^0C. A possible reason could be that the 40^0C incubation temperature is closer to the reported optimum temperature for the pectinase activity which is 50^0C and for amylase which is reported to be 45^0C (Khatri, Bhattarai, Shrestha, & Maharjan, 2015).

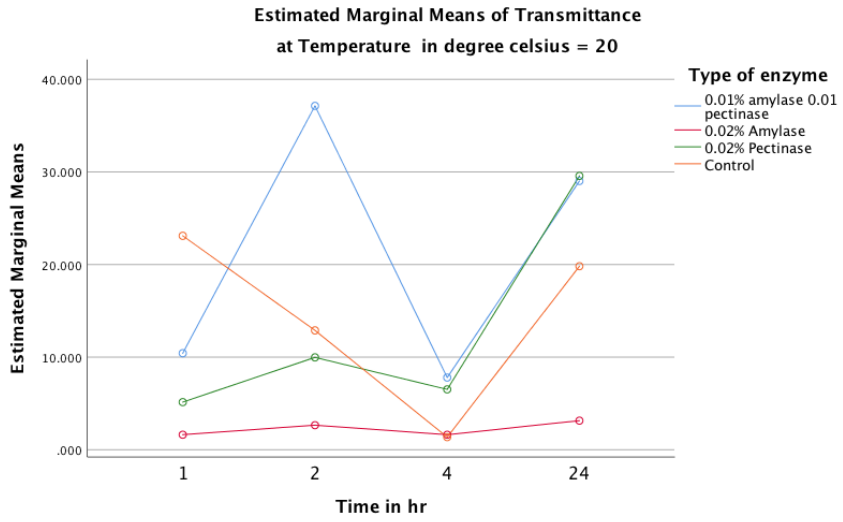

Figure 2. Interaction effect plot of incubation time and different treatments at an incubation temperature of 20^0C

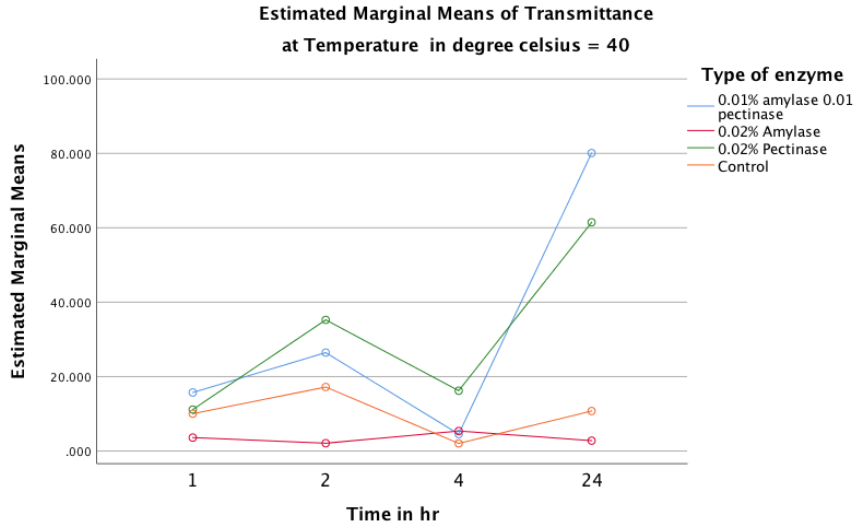

Figure 3. Interaction effect plot of incubation time and different treatments at an incubation temperature of 40^0C

The combination of the incubation time and different treatments when enzymes are added at different stages had the second-highest interaction effect. The mix of enzymes added to the pomace before juice extraction and incubated at 24-hour gave a comparable transmittance value to the pectinase enzyme added directly to the juice and incubated at 24-hour duration. Thus, if only the type of enzyme is to be considered in instances where maintaining incubation time and temperature would be difficult, a mix of pectinase and amylase added to the pomace before juice extraction will give juice with better clarity. This can be attributed to the synergistic mechanism whereby amylase prevents the agglomeration of starch with protein pectin complex leading to the prevention of haze and better clarity in the juice. As the mix of enzymes is added directly to the pomace, the synergistic effect starts in the pomace even before the juice is extracted and within 1 hour of incubation time, the clarity of the juice is higher as compared to the other treatment. However, with increasing incubation time and higher incubation temperature, the clarity of the sample where pectinase is added directly to the juice gave a comparable result.

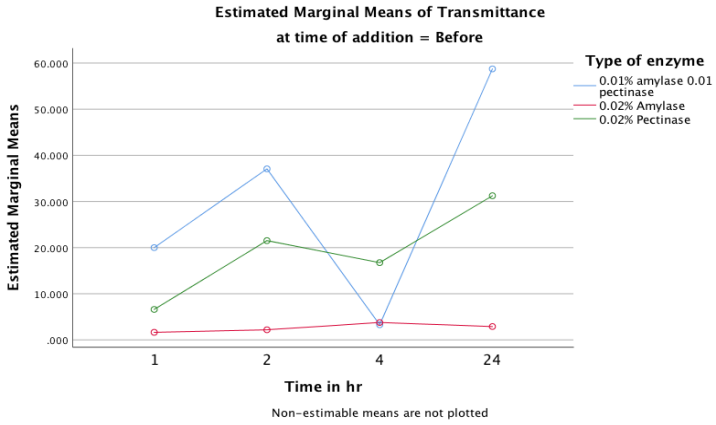

Figure 4. Interaction effect plot of incubation time and different treatment when enzyme is added to the pomace before juice extraction

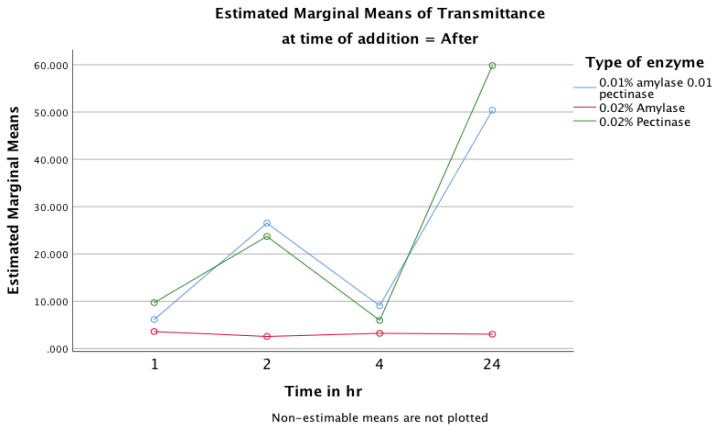

Figure 5. Interaction effect plot of incubation time and different treatments when enzyme is added to the juice

4. Conclusion

The study found that the use of enzymes did not achieve significantly different juice recovery percentages. For clarification of apple juice on a commercial scale, the process parameters must be optimized to achieve good quality clear apple juice. When it comes to the precedence of optimizing the process parameters, the most significant parameter is the type of enzyme to be used followed by the incubation time and temperature. However, since this result is based on the main effect, this case is only applicable when other process parameters such as temperature and incubation time are not considered. Thus, if the processor can only control one parameter, it would be prudent to give priority to the type of enzymes to be used and then the incubation time followed by temperature. In this study using a mix of amylase and pectinase enzyme gave the highest clarity measurement without taking into consideration the stage of enzyme addition, incubation temperature and time. Similarly, a 24-hour incubation time and the temperature maintained at 40^0C gave higher clarity juice. However, if various process parameters can be optimized to select a combination that can help achieve juice with significantly higher clarity, then the use of pectinase enzyme added directly to the juice and the mix of enzyme added to the pomace before juice extraction incubated for 24 hours at 40^0C is recommended.

Acknowledgement

The authors would like to acknowledge the staff of the National Post Harvest Centre particularly, Mrs. Chado Lham and Mrs. Tshewang Lhamo who put in extra hours in running the laboratory work. The authors would also like to thank Mrs. Dawa Choden, Mrs. Lhab Gyem and Mr. Kuenga Tenzin whose support was invaluable in running the experiment. The authors also thank the management and other staff for their valuable support during the research.

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