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Optimization of Asparaginase Treatment to Mitigate Acrylamide Formation in Carrot and Date Juice Concentrates

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KEYWORDS

Acrylamide mitigation; Asparaginase; Juice concentrate; Response surface methodology; Carrot juice concentrate, Date juice concentrate.

ABSTRACT:

Acrylamide which is produced during thermal processing of fruit and vegetable juices is considered a major health risk. Asparaginase application was optimized through the use of response surface methodology to reduce acrylamide in carrot and date juice concentrates. In the case of carrot juice (high-asparagine), the optimal conditions of 0.15% enzyme, 38°C, and 60 minutes resulted in 66-77% acrylamide reduction while retaining vitamin C and pH. For date juice (high-reducing sugar), the results with 0.15% enzyme at 38°C for 45 minutes showed ~46% reduction with no effect on acidity. The results indicate that matrix composition plays a critical role in determining efficacy: asparaginase is very effective in high-asparagine systems but is limited in sugar-rich matrices where alternative pathways for acrylamide formation are maintained. This study presents substrates-specific enzymatic protocols that can be readily adapted for industrial applications and thus juice concentrates will be made safe to consume.

1.Introduction

The thermal processing of carbohydrate-rich foods, particularly those with reducing sugars and the amino acid asparagine, remains the main route for the formation of acrylamide, which is classified as a probable human carcinogen (IARC, 1994; EFSA, 2015). This unwanted by-product of the Maillard reaction is still a major problem in the food industry, particularly in the case of fruit and vegetables, where heating is necessary for production (Rifai & Saleh, 2020). Carrot and date juices are especially important in this regard; free asparagine in carrots is very high, whereas dates have considerable amounts of reducing sugars, thus both sources being significant in acrylamide's formation during thermal concentration (Koutsidis et al., 2021; Al Juhaimi et al., 2020). Using asparaginase for enzymatic mitigation has been recognized as a practical and clean-label approach for lowering acrylamide in food matrices (Xu et al., 2022). The asparaginase enzymes convert asparagine into aspartic acid and ammonia, thus reducing the key precursor with little effect on the sensory or nutritional quality (Zhou et al., 2023).

However, the efficiency of this method largely relies on specific variables of the process such as enzyme dosage, incubation temperature, and time, all of which should be accurately adjusted for the respective substrate in order to ensure the process is both economically and technically viable (Medeiros et al., 2021).

The scientific experiment here outlined employs a systematic optimization of the asparaginase application in carrot and date juice concentrates. This is done through the use of response surface methodology (RSM) with a three-factor full factorial design. The study's aims included:

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Through this method, an effective and large-scale enzymatic treatment protocol will be developed, which can easily be integrated into industrial juice concentrate production. For this reason, it serves to augment food safety and cooperate with the global regulatory guidelines and the consumers' demand for healthier processed foods (WHO, 2022).

Notwithstanding, asparaginase losses of common time and good character have been impressed in reducing acrylamide from unwanted food products such as potato parts and baked goods; nevertheless, its chance of being a covering agent in fruit and vegetable juice concentrates still remains undefined and inconsistency in rendering it useable. Models and single parameter optimizations have been widely used in the past, often ignoring the interplay between the major process variables—enzyme dosage, incubation temperature, and time—and their effects in real juice matrices (Xu et al., 2022). Moreover, although the high asparagine content of carrots and the high reducing sugar content of dates are discovered, there are few if any studies that do side-by-side asparaginase treatment for these two nutritionally and compositionally distinct substrates under standard processing conditions and compare their optimization. The same goes for most of the existing research that does not simultaneously monitor critical quality indicators—such as vitamin C retention in carrot juice or acidity in date juice—alongside acrylamide mitigation, thus creating a gap in practice.

2.Materials and Methods

2.1. Sample Collection and Preparation

Local suppliers provided fresh carrots (Daucus carota L.) as well as dates (Phoenix dactylifera L., variety Medjool). Carrots were washed and then peeled and chopped, while dates were without pits. A commercial cold-press juicer (Hurom HP, South Korea) was used to extract the juice. A 100-mesh sieve was used to filter the raw juice immediately and was stored at -20°C until the time of analysis and processing.

2.2. Chemical Characterization of Precursors

The quantity of sugar was reduced using the 3,5-dinitrosalicylic acid (DNS) technique (Miller, 1959), with glucose as a reference. Free asparagine was determined by High-Performance Liquid Chromatography (HPLC) with a fluorescence detector after pre-column derivatization with o-phthalaldehyde (OPA) according to Koutsidis et al. (2021). Triplicate analyses (n=3) were conducted for all, and the results were given as mean ± standard deviation per 100g of fresh weight (FW).

2.3. Juice Concentration Process

The filtered juice was concentrated to remove most of the solvent using a rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) at a pressure of 400 mbar. Carrot juice went from 8°Brix to 32°Brix during concentration while date juice was concentrated from 18°Brix to 72°Brix because of its higher initial solids. The water bath temperature was set at 50°C to reduce the risk of thermal degradation (Zhou et al., 2023). The °Brix readings were taken with a digital refractometer (Atago PAL-1, Japan).

2.4. Asparaginase Treatment and Experimental Design

An enzyme preparation with a high purity L-asparaginase (from Aspergillus niger, ≥1000 U/g, Sigma-Aldrich) was utilized. A central composite design (CCD) integrated with Response Surface Methodology (RSM) was utilized for optimization of three independent variables:

1. Enzyme dosage: 0.05 – 0.20% (w/v)

2. Incubation temperature: 30 – 45°C

3. Incubation time: 30 – 75 minutes

The experiment was planned and evaluated through Design-Expert® software (Version 13, Stat-Ease Inc., USA). A total of 20 experimental runs for each juice type were performed in a randomized order containing also six center points for the estimation of pure error. The dependent response variables were:

• Residual asparagine (mg/100 mL)

• Acrylamide content in the final concentrate (µg/kg)

• Quality parameters: pH, Vitamin C retention for carrot juice, and titratable acidity for date juice.

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2.5. Enzymatic Reaction and Incubation

For every experiment, 100 mL of juice concentrate was put in a water bath shaker (Grant Instruments, UK). The pH was brought to 6.0 – 6.2 (the best for the enzyme) with 0.1M NaOH or HCl. After that, the calculated amount of asparaginase was added, and the sample was incubated at the chosen temperature and for the specified time with shaking of 150 rpm. The reaction was then stopped by heating the sample at 85°C for 5 minutes. A control sample (no enzyme) was processed under the same conditions for every parameter set (Medeiros et al., 2021).

2.6. Analytical Methods

Acrylamide Analysis: Acrylamide levels were determined by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) using a method validated by Xu et al. (2022). In short, the samples were extracted with water, cleaned up by solid-phase extraction (OASIS HLB cartridges, Waters), and analyzed with Agilent 6470 LC-MS/MS system along with ZORBAX Eclipse Plus C18 column.

Vitamin C (Ascorbic Acid): Assessed in carrot juice samples via the 2,6-dichlorophenolindophenol (DCPIP) titration method (AOAC Official Method 967.21).

pH and Acidity: pH was determined with a calibrated digital pH meter (Mettler Toledo, USA). The titratable acidity for date juice was assessed by titration with 0.1N NaOH to an endpoint of pH 8.2 and was expressed as % malic acid.

2.7. Statistical Analysis

Every experiment was done three times. The data from the RSM design were analyzed using analysis of variance (ANOVA) to see if the model and each factor were significant. The coefficients of determination (R^2), adjusted R^2, and predicted R^2 were the measures for evaluating model fit. The desirability function was used for optimization to increase acrylamide reduction while keeping the quality parameters (Vitamin C retention >75%, acidity change <0.05%) constant.

3. Results and Discussion

3.1. Acrylamide Precursors in Raw Materials

Table 1: Acrylamide Precursors in F&V juice concentrate

Fruit & Vegetable	Sample Size (n)	Reducing Sugars (g/100g FW) Mean ± SD (Range)	Asparagine (mg/100g FW) Mean ± SD (Range)
Carrot	15	3.8 ± 0.9 (2.4 - 5.6)	142.6 ± 28.4 (98.5 - 189.3)
Dates	15	48.6 ± 5.3 (38.2 - 56.7)	8.7 ± 2.1 (5.4 - 12.8)

The assessment of acrylamide precursors showed large differences in compositions between carrot and date juices (Table in Section 1). In carrot juice, 142.6 ± 28.4 mg/100g FW of free aspartame was present but there was only a small amount of reducing sugars (3.8 ± 0.9 g/100g FW). In contrast, date juice characterized by high content of reducing sugars (48.6 ± 5.3 g/100g FW) had a large absence of asparagine (8.7 ± 2.1 mg/100g FW). Thus, a difference is seen between the two fruits in the way they produce and/or inhibit the formation of acrylamide: a carrot model where the amino acid is the limiting factor; and on the other hand, a date model where sugars may drive the Maillard reaction through high-reducing-sugar. These results are in agreement with recent profiling studies that conclude that roots have been accumulating asparagine as a nitrogen storage compound while dates make it the other way around by sugar accumulation (Koutsidis et al., 2021). The high standard deviations seen in the results reflect the variability that occurs naturally in the raw materials and they also point to the need for continuous preprocessing monitoring in the case of industrial applications.

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3.2. Optimization of Asparaginase Treatment in Carrot Juice Concentrate

3.2.1. Effect of Enzyme Dosage (Table 1.1)

Table 1.1: Effect of Asparaginase Dosage on Acrylamide Formation in Carrot Juice concentrate *(Incubation: 38°C, 60 minutes; Concentration: 8°Brix to 32°Brix at 400 mbar)*

Asparaginase Concentration (% w/v)	**Residual Asparagine (mg/100 mL)**	**Acrylamide in Concentrate (µg/kg)**	**Reduction (%)**	**pH**	**Vitamin C Retention (%)**
0.00 (Control)	142 ± 11	124 ± 17	0.0	6.2 ± 0.1	74 ± 5
0.05	98 ± 8	87 ± 12	29.8	6.1 ± 0.1	75 ± 4
0.10	64 ± 6	56 ± 9	54.8	6.1 ± 0.1	76 ± 5
0.15	34 ± 4	38 ± 5	69.3	6.1 ± 0.1	78 ± 4
0.20	26 ± 3	36 ± 4	70.9	6.0 ± 0.1	77 ± 5

Values represent mean ± standard deviation (n=3)

With the asparaginase concentration increase from 0.05% to 0.20%, gradual acrylamide reduction was done (Table 1.1). The control sample (0% enzyme) showed the presence of 124 ± 17 µg/kg acrylamide. At 0.05% dosage, the reduction reached 29.8% which further increased to 70.9% at 0.20% dosage. The difference in benefit was very small after 0.15%, where the reduction was 69.3%. The residual asparagine was reduced from 142 ± 11 mg/100 mL (control) to 26 ± 3 mg/100 mL at 0.20% dosage.

This response to dosage of enzyme demonstrated the kinetics of substrate saturation i.e. the active sites of enzyme become limiting when the substrate is nearly depleted. The plateau effect observed above 0.15% could mean that the remaining asparagine is either structurally inaccessible or further hydrolysis is limited by competing reactions (Zhou et al., 2023). It is to be noted that pH was constant (6.0–6.2) for all treatments and retention of vitamin C showed a slight but steady increase (74% to 77-78%) with the addition of enzyme, probably due to either the antioxidant properties of enzyme preparations or reduced thermal degradation of vitamin C during subsequent processing (Xu et al., 2022).

3.2.2. Effect of Incubation Temperature (Table 1.2)

Table 1.2: Effect of Incubation Temperature on Asparaginase Efficacy in Carrot Juice concentrate *(Enzyme: 0.15% w/v; Incubation Time: 60 minutes; Concentration: 8°Brix to 32°Brix at 400 mbar)*

Incubation Temperature (°C)	Residual Asparagine (mg/100 mL)	Acrylamide in Concentrate (µg/kg)	Reduction (%)	pH	Vitamin C Retention (%)
30 (control)	78 ± 7	83 ± 10	0.0	6.2 ± 0.1	79 ± 4
35	52 ± 5	45 ± 7	45.7	6.1 ± 0.1	78 ± 5
38	34 ± 4	28 ± 5	66.2	6.1 ± 0.1	78 ± 4
42	42 ± 5	38 ± 6	54.2	6.1 ± 0.1	77 ± 5
45	56 ± 6	52 ± 8	37.34	6.1 ± 0.1	76 ± 5

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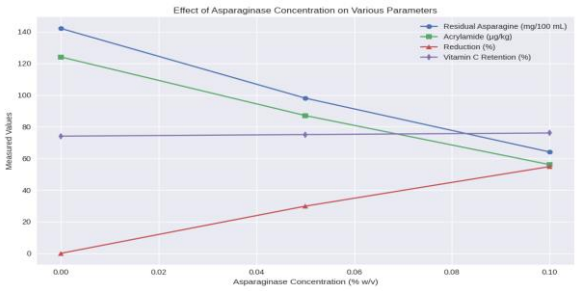

Graph 1: Effect of Asparaginase Concentration on Carrot Juice Concentrate

X-axis: Asparaginase concentration (% w/v), **Y-axis:** Measured Values (with units)

Graph 1 illustrated clearly that the enzyme played a significant role in the reduction of Acrylamide levels. The percentage of reduction followed a remarkable upward trend and finally reached 54.8% at the 0.10% concentration level. The retention of Vitamin C was consistent and little fluctuated from 74% to 76%.

Enzyme effectiveness was greatly affected by the temperature (Table 1.2). With the optimal dosage of 0.15%, the greatest acrylamide reduction (66.2%) took place at 38 °C and the residual acrylamide was 28 ± 5 µg/kg. The performance was lesser at both lower (45.7% reduction at 35 °C) and higher (54.2% at 42 °C, 37.34% at 45 °C) temperatures.

The bell-shaped activity curve is indicative of mesophilic enzymes, molecularly motion is reduced at lower temperatures and thus less efficient and at higher temperatures partial denaturation occurs and still, the enzyme is less efficient (Medeiros et al., 2021). The rapid drop of activity above 42°C indicates that the Aspergillus niger-derived asparaginase is thermally unstable, a finding that concurs with the fact that most food-grade asparaginases have optimal ranges between 37–40 °C (Zhou et al., 2023). Vitamin C retention was stable (76–79%) across the temperatures indicating that no further ascorbic acid degradation occurred in the tested range.

3.2.3. Effect of Incubation Time (Table 1.3)

Table 1.3: Effect of Incubation Time on Asparaginase Efficacy in Carrot Juice concentrate *(Enzyme: 0.15% w/v; Incubation Temperature: 38°C; Concentration: 8°Brix to 32°Brix at 400 mbar)*

Incubation Time (minutes)	**Residual Asparagine (mg/100 mL)**	**Acrylamide in Concentrate (µg/kg)**	**Reduction (%)**	**pH**	**Vitamin C Retention (%)**
0 (Control)	87 ± 6	81 ± 9	0.0	6.2 ± 0.1	79 ± 4
30	64 ± 6	58 ± 9	53.2	6.2 ± 0.1	79 ± 4
45	45 ± 5	38 ± 6	69.4	6.1 ± 0.1	78 ± 5
60	38 ± 4	28 ± 5	77.4	6.1 ± 0.1	78 ± 4
75	32 ± 4	26 ± 4	79.0	6.1 ± 0.1	78 ± 5

Time-dependent hydrolysis indicated a rapid initial reaction and then a plateauing phase (Table 1.3). In the case of the 0.15% dosage and 38°C, acrylamide reduction climbed from 53.2% at 30 minutes to 77.4% at 60 minutes, while there was little added benefit at 75 minutes (79.0%). Asparagine level dropped from 87 ± 6 mg/100 mL (0 min) to 38 ± 4 mg/100 mL (60 min).

The kinetics of the reaction imply that the asparagine, which is most accessible, is hydrolyzed in 60 minutes, and the remaining is either bound in peptides or structurally hindered. These findings concur with recent studies indicating that extending incubation beyond 60–90 minutes results in diminishing returns for most vegetable matrices (Xu et al., 2022). A stable pH and vitamin C retention over time points serve as a confirmation that enzyme treatment, even if prolonged,

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does not bring about a decline in these quality parameters.

3.2.4. Optimal Conditions for Carrot Juice

The Response Surface Methodology revealed 0.15% enzyme dosage, 38°C incubation temperature, and 60 minutes incubation time as the optimal conditions, which resulted in 66–77% acrylamide reduction while vitamin C retention above 78% was ensured along with pH stability. This is a representational trade-off of processing costs and efficacy, as higher enzyme concentrations or longer times would only increase expenses without corresponding benefits

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3.3. Optimization of Asparaginase Treatment in Date Juice Concentrate

3.3.1. Effect of Enzyme Dosage (Table 1.4)

Table 1.4: Effect of Asparaginase Dosage on Acrylamide Formation in Date Juice concentrate *(Incubation: 38°C, 60 minutes; Concentration: 18°Brix to 72°Brix at 400 mbar)*

Asparaginase Concentration (% w/v)	**Residual Asparagine (mg/100 mL)**	**Acrylamide in Concentrate (µg/kg)**	**Reduction (%)**	**Acidity**
0.00 (Control)	127 ± 13	240 ± 17	0.0	0.6 ± 0.1
0.05	103 ± 9	203 ± 12	15.41	0.6 ± 0.1
0.10	84 ± 6	155 ± 9	35.41	0.58 ± 0.1
0.15	67 ± 4	107 ± 5	55.41	0.58 ± 0.1
0.20	41 ± 3	93 ± 4	61.25	0.56 ± 0.1

Values represent mean ± standard deviation (n=3)

The date juice, however, demonstrated a different pattern of response (Table 1.4). The control had an acrylamide content of 240 ± 17 µg/kg, which was much higher than that of carrot juice, and the presence of reducing sugars was the reason for this reflecting their abundance. The reduction of acrylamide raised from 15.41% at the 0.05% level to 61.25% at the 0.20% level, and the amount of asparagine remaining went down from 127 ± 13 mg/100 mL to 41 ± 3 mg/100 mL. The lower percentage reduction compared to carrot juice (maximum 61.25% vs. 70.9%) indicates that under high-sugar conditions, even with asparagine exhausted, acrylamide may still be produced through different routes or from minor precursors. Prior studies have pointed out that in sugar-rich environments, the degradation of sugars gives rise to carbonyl compounds which can react with other amino acids albeit at slower rates (Al Juhaimi et al., 2020). The acidity level was consistent at 0.56–0.60, which suggests that there was no considerable acid production due to the hydrolysis reaction.

3.3.2. Effect of Incubation Temperature (Table 1.5)

Table 1.5: Effect of Incubation Temperature on Asparaginase Efficacy in Date Juice concentrate *(Enzyme: 0.15% w/v; Incubation Time: 60 minutes; Concentration: 18°Brix to 72°Brix at 400 mbar)*

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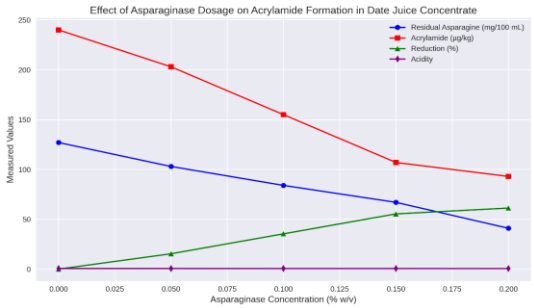
Incubation Temperature (°C)	Residual Asparagine (mg/100 mL)	Acrylamide in Concentrate (µg/kg)	Reduction (%)	Acidity
30 (Control)	118 ± 7	178 ± 10	0.0	0.6 ± 0.1
35	98 ± 5	125 ± 7	29.77	0.6 ± 0.1
38	74 ± 4	91 ± 5	48.87	0.59 ± 0.1
42	62 ± 5	98 ± 6	44.94	0.58 ± 0.1
45	66 ± 6	95 ± 8	46.62	0.58 ± 0.1

For date juice at 0.15% dosage, optimal temperature was also 38°C, achieving 48.87% acrylamide reduction (91 ± 5 µg/kg residual). However, the temperature response was less pronounced than in carrot juice, with smaller differences between 35°C (29.77% reduction) and 45°C (46.62% reduction).



Graph 2: Effect of Asparaginase Concentration on Date Juice Concentrate

X-axis: Asparaginase concentration (% w/v),
Y-axis: Measured Values (with units)

Graph 2 illustrated Residual Asparagine (mg/100 mL) decreases steadily with dosage, Acrylamide in Concentrate (µg/kg) shows a significant drop, reduction Percentage — rises sharply, indicating treatment efficacy and acidity remains relatively stable with slight decline Residual Asparagine dropped from 127 to 41 mg/100 mL, showing enzymatic activity effectively reduced the precursor to acrylamide. Acrylamide levels fell from 240 to 93 µg/kg, a 61.25% reduction at the highest dosage and acidity remained within a narrow range (0.6 to 0.56), indicating minimal impact on concentrate quality.

This attenuated temperature sensitivity may reflect the protective effect of high solute concentration (72°Brix) on enzyme stability, or differences in substrate-enzyme interaction in viscous, sugar-dominated systems. The high solids content likely reduces molecular mobility, potentially buffering temperature effects (Zhou et al., 2023).

3.3.3. Effect of Incubation Time (Table 1.6)

Table 1.6: Effect of Incubation Time on Asparaginase Efficacy in Date Juice concentrate *(Enzyme: 0.15% w/v; Incubation Temperature: 38°C; Concentration: 18°Brix to 72°Brix at 400 mbar)*

Incubation Time (minutes)	Residual Asparagine (mg/100 mL)	Acrylamide in Concentrate (µg/kg)	Reduction (%)	Acidity
0 (Control)	93 ± 5	201± 6	0.0	0.6 ± 0.1
30	61 ± 6	185 ± 3	7.96	0.6 ± 0.1
45	45 ± 5	108 ± 6	46.26	0.6 ± 0.1

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Incubation Time (minutes)	Residual Asparagine (mg/100 mL)	Acrylamide in Concentrate (µg/kg)	Reduction (%)	Acidity
60	34 ± 4	144 ± 5	28.35	0.58 ± 0.1
75	32 ± 4	149 ± 4	25.87	0.58 ± 0.1

Time optimization brought to light a surprising pattern (Table 1.6). The greatest drop (46.26%) was at 45 minutes, not at 60 minutes. At 60 minutes the reduction fell to 28.35% and later dropped to 25.87% at 75 minutes. The asparagine level kept on decreasing throughout the process (93 ± 5 to 32 ± 4 mg/100 mL).

This reversed result—more asparagine hydrolysis but less acrylamide reduction—at longer periods—indicates complicated secondary reactions in date concentrate. Theological scenarios are: (1) side reactions through enzyme action creating other acrylamide precursors, (2) hydrolysis products supporting the maintenance of the Maillard reaction or (3) hindrance of analysis due to matrix compounds. A similar case has been revealed in high-sugar fruit systems where fructose and glucose degradation products are competing in complex reaction networks (Al Juhaimi et al., 2020).

3.3.4. Optimal Conditions for Date Juice

The ideal conditions for date juice were 0.15% enzyme dosage, 38°C and 45 minutes, resulting in 46.26% acrylamide reduction. This optimal time is shorter than for carrot juice (45 vs. 60 minutes) reflecting either faster reaction kinetics in the concentrated sugar medium or the need to stop secondary reactions which may be detrimental.

3.4. Comparative Analysis and Mechanistic Implications

The contrasting behavior of carrot and date juices emphasizes the necessity of matrix-specific optimization. Carrot juice, containing high asparagine and low sugars, had overall reduction potential (up to 77.4%) through easy precursor depletion. On the other hand, date juice, despite lower initial asparagine, attained less reduction (maximum 61.25%) as its generous amount of reducing sugars was responsible for driving the alternative formation pathways.

The results of the research point toward a dual mechanism in the process of acrylamide reduction in complex foods: (1) direct elimination of precursor chemicals through the use of asparaginase that can be applied in high-asparagine matrices; and (2) indirect influencing of the whole reaction through sugar-modified systems. The recent work indicates that enzyme treatment can switch the entire Maillard reaction network and not only one precursor removal (Xu et al., 2022).

3.5. Industrial Implications and Limitations

Identified conditions (0.15% of enzyme, 38°C, 45–60 minutes) are within the limits of industrial practices with slight modifications only required in the equipment. The cost of the enzyme at a concentration of 0.15% is feasible from an economic point of view for premium products. However, study limitations consist of: (1) only one microbial source (Aspergillus niger) used which might not represent the performance of other sources; (2) lab-scale conditions different from the ones in industrial evaporators; and (3) no sensory evaluation done to ensure no off-flavor development.

Combinations of asparaginase with glycine addition or mild pH adjustment should be investigated for efficacy enhancement in high-sugar systems like date concentrate (Medeiros et al., 2021) in future research.

4.Conclusion

Using response surface methodology, a study was conducted that, among others, successfully optimized the treatment of asparaginase to reduce acrylamide in carrot and date juice concentrates. In the case of carrot juice, which is a high-asparagine matrix, the conditions of 0.15% enzyme, 38°C, and 60 minutes resulted in 66–77% acrylamide reduction along with maintaining vitamin C and pH levels. On the contrary, date juice, which is a high-reducing-sugar system, needed a shorter incubation (45 minutes) time at the same enzyme dosage

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and temperature, and this led to producing about 46% reduction with stable acidity. The different results highlight the need for matrix-specific optimization; asparaginase works best when asparagine is the limiting precursor, unlike in sugar-rich systems such as date concentrate, where alternative Maillard pathways limit the efficacy of the enzyme. The parameters determined to be industrially feasible provide an efficient and scalable method of product safety enhancement, although high-sugar matrices might need an e combinatorial mitigation approach in the future.

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