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SIMULTANEOUS EXTRACTION OF RICE MALT AMYLASES AND PROTEASES

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ABSTRACT

Dehusked unpolished rice grains ("Mottaikaruppan" variety) were germinated in a moistened bag wetted with 0.15gL^{-1} $Na_2S_2O_3$ in dark at 30°C for 4 days and conditions were optimized to extract endogenous amylases and proteases. Extractants such as distilled water or 0.01M acetate/acetic acid buffer (pH 5.0) or 9.0gL^{-1} NaCl or 1.0gL^{-1} $CaCl_2$ or 0.01M acetate/acetic acid buffer (pH 5.0) - 9.0gL^{-1} NaCl or 0.01M acetate/acetic acid buffer (pH 5.0) -1.0gL^{-1} $CaCl_2$ or 9.0gL^{-1} NaCl - 1.0gL^{-1} $CaCl_2$ were used to extract malt amylase. The best extractant for rice malt amylases was NaCl and 10gL^{-1} was the best concentration. When malt amylases were repeatedly extracted with 10gL^{-1} NaCl for six times, the first two extracts contained the same activity. Malt proteases were extracted with distilled water or 10gL^{-1} NaCl or 1% (v/v) glycerol or 0.01M phosphate buffer (pH 7.0) or 0.01M phosphate buffer (pH 7.2) or 0.01M phosphate buffer (pH 7.2) - 10gL^{-1} NaCl and, 0.01M phosphate buffer (pH 7.2) extracted highest activity. The most suitable pH for the extraction of malt proteases with 0.01M phosphate buffer was 7.5 and the phosphate buffer concentration was 0.03M. When malt proteases were repeatedly extracted with 0.03M phosphate buffer pH 7.5 for five times the first extract contained the highest activity. For simultaneous extraction of malt amylase and malt protease, 0.01M phosphate buffer (pH 7.4) was the best extractant and 50°C was the most suitable temperature. Combined effect of detergent 0.1% (v/v) (Triton X-100) and temperature on the extraction of malt enzymes were studied. Triton X-100 (0.1%, v/v) improved the extraction of amylases and proteases by 11.5 and 1.8% respectively and 50°C was the best temperature. When the pH stabilities of malt enzymes were studied in presence of 0.1% (v/v) (Triton X-100)- 0.01M phosphate buffer pH 7.4 at 30°C, malt amylases were stable at pH 4.5 and 8.0 and malt proteases were stable at pH 4.0 and 7.5.

Key words: Malt Amylases; Malt Proteases; Rice Malt Enzymes; Endo Enzymes; Extraction of Enzymes

INTRODUCTION

Malting of cereals is in industrial use for several food preparations (Finney, *et al.*, 1972). The malt enzymes could also be used as sources of amylases and proteases (Arasaratnam, *et al.*, 1998). To use these enzymes as sources, they need to be extracted from the malted cereal flour. The extraction process involves the leaching of the enzyme (Ramakrishna, *et al.*, 1982) in suitable solvent and negligible reference is available in the literature on the extraction of enzymes from malted cereals. In general a lot of work has been done for the extraction of enzymes from moldy medium obtained by solid state fermentation. The extractants used are cold water (Ramakrishna, *et al.*, 1982; Ramesh and Lonsane, 1988), 1% NaCl (Ramesh and Lonsane, 1988), glycerol containing distilled water (Ramesh and Lonsane, 1988), buffer (Ramesh and Lonsane, 1988) and tap water (Ramesh and Lonsane, 1988; Ghildyal, *et al.*, 1991) at different temperatures. During the studies on malting of a local variety of rice ("Mottaikaruppan" variety) the scant information on the factors affecting the recovery of the enzymes from malted rice powder became apparent and hence were studied in detail. The individual and simultaneous extractions, of the endogenous enzymes of the malted rice were studied in view of utilizing the malted rice in food industry.

MATERIALS AND METHODS

Materials

Dehusked unpolished rice ("Mottaikaruppan" a local land variety to the Northern region of Sri Lanka) was purchased from the local market and used as raw material. Soluble starch (AR), Triton X-100 and casein (sodium salt) were from Sigma Chemical Company (USA). All the other chemicals used were of analytical grade. All the experiments were performed in triplicate and the average values are presented.

Simultaneous Extraction of Rice Malt Amylases and Proteases

Malting of unpolished rice and preparation of malted rice powder

Dehusked unpolished rice grains ("Mottaikaruppan" variety) were steeped in distilled water containing $0.1gL^{-1}$ $Na_2S_2O_5$ for 12h and germinated in a moistened bag wetted with $0.15gL^{-1}$ $Na_2S_2O_5$ while in dark at 30°C. Germination of the dehusked unpolished rice grains was arrested on the 4th day, dried at 35°C to a constant weight and powdered at room temperature in a domestic grinder. This powder of malted rice was used for the extraction and separation of enzymes and characterization of malt enzymes.

Determination of malt amylase and protease activities

The malted rice extract supernatant obtained was analyzed for amylase (Bernfeld, 1951) and protease (Anson, 1938) activities.

Amylolytic Unit (AU) of rice amylase

One unit of rice amylase is the amount of enzyme, which releases 1.0µmole of glucose in one minute at 60°C and pH 5.0 from $40gL^{-1}$ soluble starch.

Proteolytic Unit (PU) of rice protease

One unit of rice protease is the amount of enzyme that liberates 1.0µmole of tyrosine from $10gL^{-1}$ casein in one hour at 50°C and pH 7.2.

Optimizing the conditions for the extraction of rice malt amylase

Determining a suitable extractant

Malted rice powder (1.0g) was mixed with 10.0mL of either distilled water or 0.01M acetate/acetic acid buffer (pH 5.0) or $9.0gL^{-1}$ NaCl solution or $1.0gL^{-1}$ $CaCl_2$ solution or 0.01M acetate/acetic acid buffer (pH 5.0) - $9.0gL^{-1}$ NaCl solution or 0.01M acetate/acetic acid buffer (pH 5.0) -$1.0gL^{-1}$ $CaCl_2$ solution or $9.0gL^{-1}$ NaCl - $1.0gL^{-1}$ $CaCl_2$ solution. The suspensions were mixed well at 30°C for 15minutes. The extracts were centrifuged (MSE bench centrifuge) at speed 3000rpm for 5 minutes. The supernatants were analyzed for malt amylase activity (Bernfeld, 1951).

Effect of sodium chloride concentration on the extraction of rice malt amylase

Malted rice powder (1.0g) was mixed with 10.0mL of NaCl solution of varying concentrations (0, 4, 8, 9, 10, 11, 12, 14, 16 and $20gL^{-1}$) and the experiment was proceeded as described above.

Repeated extraction of malt amylase from malted rice powder

Malted rice powder (1.0g) was extracted with 10.0mL portions of $10gL^{-1}$ NaCl as described above. After the removal of the supernatant of the 1st extract, amylase in the residue was again extracted with 10.0mL of $10gL^{-1}$ NaCl for five times. Supernatants obtained in all six extracts were analyzed for amylase activity.

Optimizing the conditions for the extraction of rice malt protease

Determining a suitable extractant

Malted rice powder (1.0g) was mixed with 5.0mL of either distilled water or $10gL^{-1}$ NaCl solution or 1% (v/v) glycerol solution or 0.01M phosphate buffer (pH 7.0) or 0.01M phosphate buffer (pH 7.2) or 0.01M phosphate buffer (pH 7.2) - $10gL^{-1}$ NaCl solution at 30°C for 15minutes. The extracts were centrifuged (MSE bench centrifuge) at speed 3000rpm for 5 minutes. The supernatants obtained were analyzed for malt protease activity.

Effect of pH on the extraction of rice malt protease

Malted rice powder (1.0g) was mixed with 5.0mL of 0.01M phosphate buffer of varying pH values ranging from 6.0 – 8.0 and the experiment was proceeded as described above.

Phosphate ion concentration on malt protease extraction

Malted rice powder (1.0g) was mixed with 5.0mL of phosphate buffer (pH 7.5) of varying concentrations (0.00, 0.005, 0.01, 0.02, 0.025, 0.03, 0.04 and 0.05M) and the experiment was proceeded as described above.

Repeated extraction of malt protease from malted rice powder

Malt protease in malted rice powder (1.0g) was extracted with 5.0mL portions of 0.03M phosphate buffer (pH 7.5) as described above. After the removal of supernatant of the 1st extract, the proteases in the residue

were extracted for three times. Supernatants obtained in each four extraction were analyzed for protease activity.

Optimizing the conditions for simultaneous extraction of malt amylase/s and protease/s from malted rice powder

Effect of pH on the extraction of malt enzymes

Malted rice powder (1.0g) was mixed with 10.0mL of buffer solutions of varying pH values ranging from 3.0-8.0 (for pH 3.0-7.0, 0.01M citrate-0.02M phosphate buffer and for pH 7.0-8.0, 0.01M phosphate buffer) at 30°C for 30minutes. The supernatants of the extracts (centrifuged in MSE bench centrifuge at speed 3000rpm for 5 minutes) were analyzed for malt amylase and malt protease activities.

Effect of temperature on the extraction of malt enzymes

Rice malt powder (1.0g) was mixed with 0.01M phosphate buffer (10.0mL, pH 7.4) at different temperatures (30, 35, 40, 45, 50 and 60°C) for 30min and the experiment was preceded as described above.

Combined effect of detergent (Triton X-100) and temperature on the extraction of malt enzymes

Malted rice powder (1.0g) was mixed with 0.01M phosphate buffer (10.0mL, pH 7.4) or with 0.01M phosphate buffer (10.0mL, pH 7.4) containing 0.1% (v/v) Triton X-100. Then, they were incubated at different temperatures (45, 48, 50 and 55°C) for 30minutes. The experiment was preceded as said above.

Effect of repeated extraction of malt enzymes and soluble proteins from malted rice powder under the optimized conditions

(i) In the absence of Triton X-100

Malted rice powder (1.0g) was extracted with 10.0mL portions of 0.01M phosphate buffer (pH 7.4) at 50°C for 30minutes. This repeated extraction was carried out for four times as described above. The suspensions obtained were analyzed for soluble protein content and amylolytic & proteolytic activities.

(ii) In the presence of Triton X-100

The above experimental procedure was followed. Here the extraction was carried out with 0.01M phosphate buffer (pH 7.4) containing 0.1% (v/v) Triton X-100 instead of 0.01M phosphate buffer (pH 7.4).

pH stability of malt enzymes of the malted rice powder

Malt amylase and protease extracted under optimized conditions in presence of 0.1% (v/v) Triton X-100 was added with 0.02% sodium azide and the pH was adjusted to different values (ranging from 3.0 to 8.0) with either 0.5N HCl or 0.5N NaOH. Samples stored at 30°C were analyzed for amylolytic and proteolytic activities on the 3rd day.

RESULTS AND DISCUSSION

Extraction of malt amylase of malted rice powder

Determining a suitable extractant for malt amylases from malted rice powder

Different solutions were used to extract malt amylase from malted rice powder of dehusked unpolished "Mottaikaruppan" rice. The highest relative extraction efficiency of 223.9Ug^{-1} dry matter for amylolytic activity was obtained with 9.0gL^{-1} NaCl followed with 0.01M acetate/acetic acid buffer (pH 5.0)- 9.0gL^{-1} NaCl and distilled water. The least value (161.0Ug^{-1} dry matter) was obtained with 0.01M acetate/acetic acid buffer (pH 5.0)-1.0gL^{-1} $CaCl_2$ (Figure 1). The extractants containing $CaCl_2$ were not efficient in the extraction of amylases while the extraction with distilled water was better than that in presence of $CaCl_2$ Therefore $CaCl_2$ was avoided for the extraction of amylase even though Ca^{2+} is essential for the stability and activity of amylases (Hagenimana, *et al.*, 1994).

Effect of sodium chloride concentration on the extraction of rice malt amylases

When the concentration of the NaCl was increased from 0 to 10gL^{-1}, amylase activity extracted was increased and the relative amylolytic activity was highest in the malted rice extract supernatant obtained with 10gL^{-1} NaCl solution (Figure 2). In view of improving the amount of malt amylase extraction, repeated extraction with 10gL^{-1} NaCl solution was carried out.

Effect of repeated extraction of malt amylases from malted rice powder

The repeated extraction was carried out for 6 times. In the 1st and 2nd extracts 60.0 and 19.5% of the total activities were obtained (Table 1). Subsequent extractions showed gradual decrease in activity. Final extract (Extract 6) gave the amylolytic activity of $7.75Ug^{-1}$ dry matter, which was 2.0% of the total activity extracted.

Extraction of malt proteases of malted rice powder

Extraction of malt proteases from malted rice powder with different solutions

Highest ($0.139Ug^{-1}$ dry matter) proteolytic activity of rice malt protease was obtained with 0.01M phosphate buffer (pH 7.2) followed with 0.01M phosphate buffer (pH 7.0). Least protease extraction ($0.086Ug^{-1}$ dry matter) was obtained with 0.01M phosphate buffer (pH 7.2)-$10gL^{-1}$ NaCl solution (Figure 3). This study revealed that 0.01M phosphate buffer (pH 7.2) was the best extractant for malt proteases. As opposed to amylase extraction, NaCl decreased protease extraction. 0.2M acetic acid was the most efficient solvent to solubilize the proteases in wheat flour; among water, 0.2M acetate buffer (pH 3.8) and 0.2M acetic acid solutions (Wang and Grant, 1969). For the extraction of acid proteases from mouldy bran, highest relative extraction efficiency was obtained with 0.01M phosphate buffer (pH 6.5) and distilled water (Senthuran, 1997). The results indicated that the pH of 0.01M phosphate buffer has influenced the extraction of malt proteases.

Effect of pH on the extraction of rice malt proteases

Since the pH of phosphate buffer showed positive effect on the extraction of malt proteases from malted rice powder, it was decided to study the effect of pH on the extraction of malt proteases, by varying the pH range from 6.0 to 8.0 using 0.01M phosphate buffer. Increase in protease activity was observed with increase in pH from 6.0 to 7.5, where the highest activity of $0.154Ug^{-1}$ dry matter was achieved with 0.01M phosphate buffer (pH 7.5) as extractant (Figure 4). Further increase in pH decreased the enzyme extraction. Here it is important to note that the enzyme activity measurement was carried out at pH 7.2 in 0.01M phosphate buffer. Large portion of the wheat flour proteinases were readily extracted by water adjusted to pH 8.0 or acetate buffer (pH 3.8), but not by distilled water (at pH 5.8, McDonald and Chen, 1964). Since, pH has an effect on the extraction of rice malt proteases, it was decided to study the influence of phosphate ion concentration on the extraction at pH 7.5.

Effect of phosphate ion on the extraction of rice malt proteases

Extraction of rice malt powder with varying concentrations (0-0.05M) of phosphate buffer (pH 7.5) at 30°C was studied (Figure 5). When the concentration of the phosphate buffer (pH 7.5) was increased from 0 to 0.03M, the malt protease activity in the supernatant increased and attained the optimal value of $0.157Ug^{-1}$ dry matter with 0.03M phosphate. It is also important to note that the malt proteases extracted in the absence of phosphate ions was less when compared to that in the presence of phosphate ions. Hence, it can be assumed that phosphate ions have a releasing or solubilizing effect on the malt protease extracted from malted rice powder. Therefore 0.03M phosphate buffer (pH 7.5) was selected to extract malt proteases at 30°C. Extraction of acid proteases from mouldy bran was not affected by the concentration (0-0.05M) of phosphate buffer (pH 6.5) at 30°C (Senthuran, 1997). Under the optimized conditions, it was decided to carry out multiple step extractions of malt proteases from malted rice powder in order to increase the amount of enzyme extracted.

Effect of repeated extraction of malt proteases from malted rice powder

Since malt proteases extracted in one step procedure gave was very less activty, it was decided to carry out repeated extraction. Protease in malted rice powder was extracted four times with 0.03M phosphate buffer (pH 7.5) at 30°C and occasional shaking for 15min. First extract showed 63.2% of the total protease activity (Table 1). In the second and third extracts, 25.2 and 9.0% of the total activities were obtained. Subsequent extracts showed very small activity. Single extraction of the wheat flour with acetate buffer would solubilize less than one-half of the proteolytic activity found in a flour suspension. Repeated extractions of the residue could solubilize additional activity as well as additional proteins (Wang and Grant, 1969). These observations agree with our results too.

Optimizing the conditions for the simultaneous extraction of rice malt amylase/s and protease/s

Influence of pH on the extraction of malt enzymes

As the best extractants for rice malt amylase and for malt protease were different, it was decided to optimize the conditions for the simultaneous extraction of both malt enzymes. Initially the effect of pH on simultaneous extraction of both enzymes (from pH 3.0 to 8.0) was studied. Amylase extraction showed a sharp increase with pH from 3.0 to 4.5 giving the highest extraction at pH 4.5 ($207.03Ug^{-1}$ dry matter) and 30°C (Figure 6). Increase in pH by 3 units from 5.0 – 8.0 resulted in $22.0Ug^{-1}$ dry matter drop in enzyme extraction. Two pH units below pH 5.0 has resulted in $68.57Ug^{-1}$ dry matter drop in enzyme extraction. When the pH was increased highest extraction was $0.145Ug^{-1}$ dry matter obtained at pH 7.4 and 30°C of (Figure 6). The optimum pH values for the extraction of malt amylase(s) and malt protease(s) were 4.5 and 7.4 respectively. If the extraction of malt enzymes is carried out at pH 4.5 (optimum pH for the extraction of malt amylases), the amount of malt protease extracted will be very low and would be 25.9% of that extracted at pH 7.4. But if the extraction is carried out at pH 7.4, (which is the pH optimum for the extraction of malt proteases) a considerable amount of malt amylases ($189.97Ug^{-1}$ dry matter, 92.1% of that extracted at pH 4.5) could be extracted. Hence, it was decided to select pH 7.4 for simultaneous extraction of malt amylase(s) and malt protease(s) from malted rice powder. Optimum temperature for the simultaneous extraction of malt enzymes was studied.

Effect of temperature on the extraction of malt enzymes

The extraction of malt amylases increased with increasing temperature from 30 to 50°C, and showed the highest extraction ($213.28Ug^{-1}$ dry matter) at 48°C with 0.01M phosphate buffer (pH 7.4) (Figure 7). Optimum temperature for the extraction of malt proteases was also 50°C. Increase in enzyme extraction with temperature could be due to the increase in the solubility of enzyme with temperature. Decreased activity at high temperatures resulted due to the denaturation of the enzyme protein by heat. Since, the optimum temperatures for the extraction of both the enzymes were close to each other, it was decided to fix the temperature as 50°C, for the simultaneous extraction of malt enzymes. In order to improve the enzyme extraction, the effect of temperature in presence of detergent was studied.

Effect of temperature in presence of detergent on the extraction of malt enzymes

Earlier experiment revealed that simultaneous extraction carried out at 50°C and pH 7.4 yielded considerable amounts of malt enzymes (proteases and amylases) from malted rice powder. Triton X-100 (0.1%, v/v) was incorporated into the extractant to improve the solubility of enzymes (proteins) in the malted rice powder. When the temperature was increased from 45 to 48°C, the extraction of rice malt amylases increased both in the test (with 0.1% (v/v) Triton X-100) and control (without Triton X-100) (Figure 8). But in all cases, the amount of extraction in the test samples was slightly higher than that in the control samples. Extraction of rice malt proteases increased with increasing temperature and showed the optimum extraction at 50°C, both in the test and control samples. Here also, slightly higher extraction values were observed with the test samples. These results showed that incorporation of Triton X-100 improved simultaneous extraction of malt enzymes. The malt amylases and malt proteases extraction increased up to 16.66% at 48°C and 11.26% at 50°C respectively. Since enzyme extraction was increased in all test samples, action of detergent is independent of temperature. Anionic detergent usually improves the solubilization of proteins (Putnam, 1948). When rice proteins are extracted detergent improved the extraction efficiency (MacIntyre and Kymal, 1956). Another important point to be noted is that, the optimum extraction temperature for the two enzymes is not the same. In spite of slight difference in the extraction temperature of the two rice malt enzymes, 50°C was selected as a suitable temperature for the simultaneous extraction of malt enzymes from malted rice powder for further studies. Since repeated extraction has an influence on the extraction of malt enzymes individually under the optimized conditions, simultaneous repeated extraction of malt enzymes under selected conditions were carried out.

Influence of repeated & simultaneous extraction of malt enzymes of malted rice powder under the optimized conditions

Malt amylases and proteases of malted rice powder were extracted with 0.01M phosphate buffer (pH 7.4) with and without Triton X-100, at 50°C for five times. Incorporation of Triton X-100 into the extraction medium increased the extraction of malt amylases and proteases and soluble proteins (Table 2). In presence of Triton X-100, 80.0 and 11.2% of amylase activities present in the suspension were extracted in the 1st and 2nd extractions. Similarly, 72.8 and 9.4% of amylase activity present in the suspension was extracted in the 1st and 2nd extractions of the control experiment. Obviously these results indicated that addition of Triton X-100 enhanced the extraction of rice malt amylases. Total amylase activity obtained in the supernatants; in

presence and absence of Triton X-100 were 99.4 and 87.9% respectively. Total amylase activity present in the suspension (test sample) of malted rice powder was almost completely extracted in the five supernatants of the repeated extraction carried out under the optimized conditions. These results indicated that extraction of malt enzymes are influenced by the addition of Triton-X-100. In presence of Triton X-100, total soluble proteins extracted in the five exrtractants was 19.0% of the total soluble proteins present in the malted rice powder suspension. In the control experiment, 20.7% of the total soluble protein was extracted. In presence and absence of Triton X-100, protease activity observed in the suspension was 0.1892 and 0.1801Ug^{-1} dry matter respectively under the optimized conditions. Both in the test and control, large amount of proteases was extracted in the 1st and 2nd extractions. Subsequent extractants showed very little amount of activity. Total protease activity obtained in the extracts of repeated extraction in presence and absence of Triton X-100 was 99.8 and 98.0% respectively. Here the protease enzyme was also completely recovered by the repeated extraction carried out in presence and absence of Triton X-100, under the optimized conditions. Hence, these optimized extraction conditions applied for repeated extraction was most suitable for the extraction of malt enzyme proteins. Storage stability of the enzymes in presence of Triton X-100 was studied.

Effect of pH on the storage stability of rice malt enzymes

Since the simultaneous extraction of amylase and protease from rice malt powder was efficient with 0.01M phosphate buffer containing 0.1% (v/v) Triton X-100 at pH 7.4 and 50°C, the stabilities of the enzymes at different pH values (ranging from 3.0-8.0) in presence of Triton-X-100 was studied at 30°C for 3 days. The amylase and protease activities retained on the 3rd day are presented as relative activities (Figure 9). When the pH was increased from 3.0 to 4.5 the relative malt amylase activity retained was increased and was higher at pH 4.5 (88.7%). The relative activity of the amylase retained again was high from pH 7.0 to 8.0. At pH 8.0 the relative activity retained was 91.2% of that of the initial activity. These results suggest that rice malt has more than one amylase and the malt amylases of the "Mottaikaruppan" rice are stable over a wide range of pH, retaining 86.1% activity at pH 4.0 and 91.2% at pH 8.0. Hence, most suitable pH values for the storage of malt amylases at 30°C are pH 4.5 (acidic) and 8.0 (alkaline). Sweet potato α-amylase retained its activity in the pH range from 5.0 to 9.0 at 4°C (MacIntyre and Kymal, 1956). When the pH was increased from 3.0 to 4.0, the stability of proteases increased and retained 95.7% relative activity at pH 4.0. Increase in pH from 4.0 to 6.0 led to sharp decrease in the stability of protease. Stability of the enzymes again increased from pH 6.0 to 7.5. In this experiment, rice malt proteases retained more than 90.0% of their activity at two distinct pH values namely at pH 4.0 and 7.5. In between these two pH values the stability of the enzyme sharply dropped. These results indicated that the malted rice contains more than one type of protease.

CONCLUSION

Extraction of malt amylases and proteases from malted rice individually showed variation in the extractants. The extraction of amylase in presence of 10gL^{-1} NaCl was very high where protease extraction was low in presence of 10gL^{-1} NaCl. Simultaneous extraction of amylase and protease with 0.01M phosphate buffer (pH 7.4) at 50°C has shown that the extraction of malt amylase and protease were 88.5 and 30.5% of their extractions under the optimized conditions of the individual enzymes. Further Triton-X-100 has improved the extraction of malt amylases and proteases by 3.9 and 65.7% respectively. The stability studies revealed that the rice malt powder contains more than one type of amylase and protease. Further investigations are necessary to prove the presence of more than one fraction of malt amylases and proteases.

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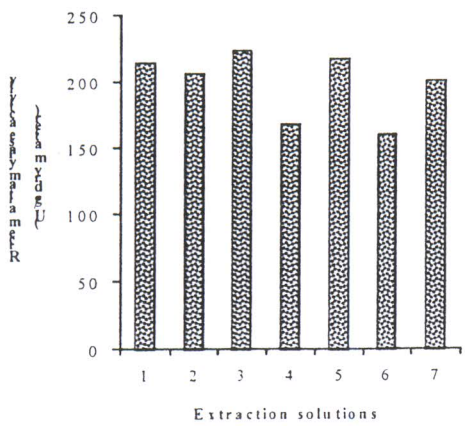

Figure 1: Effect of different solutions on the extraction of malt amylases from malted rice powder at 30°C with occasional shaking for 15 minutes. (1), Distilled water; (2), 0.01M Acetate/Acetic acid buffer (pH 5.0); (3), 9.0gL^{-1} NaCl; (4), 1.0gL^{-1} $CaCl_2$; (5), 0.01M Acetate/Acetic acid buffer (pH 5.0)- 9.0gL^{-1} NaCl; (6), 0.01M Acetate/Acetic acid buffer (pH 5.0)-1.0gL^{-1} $CaCl_2$; (7), 9.0gL^{-1} NaCl-1.0gL^{-1} $CaCl_2$.

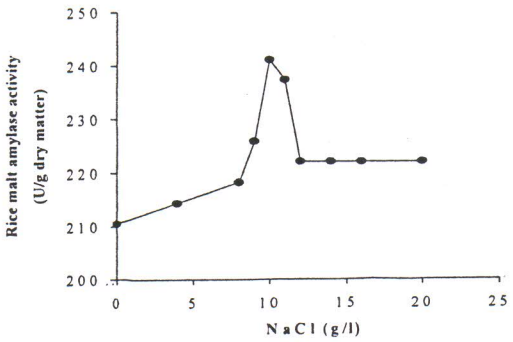

Figure 2: Effect of different concentrations of sodium chloride on the extraction of rice malt amylases.

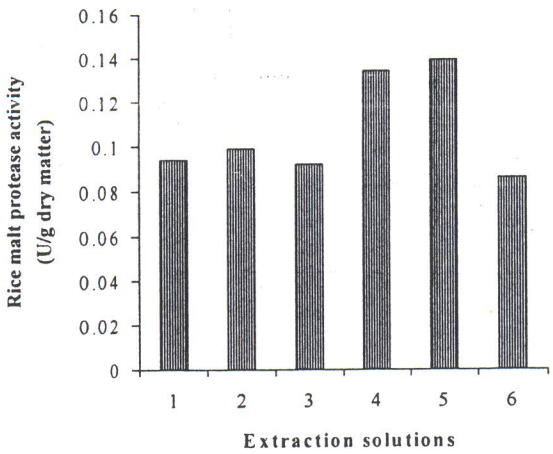

Figure 3: Effect of different solutions on the extraction of rice malt proteases. (1), Distilled water; (2), $10gL^{-1}$ NaCl; (3), 1.0% (v/v) Glycerol; (4), 0.01M Phosphate buffer (pH 7.0); (5), 0.01M Phosphate buffer (pH 7.2); (6), 0.01M Phosphate buffer (pH 7.2) - $10gLl^{-1}$ NaCl.

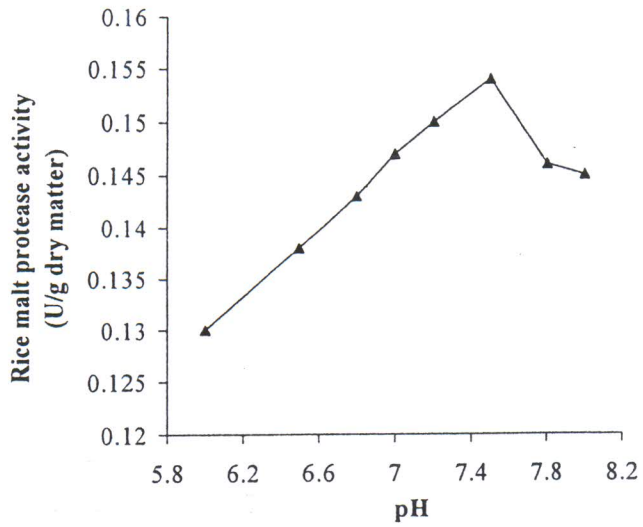

Figure 4: Effect of pH on the extraction of malt proteases at 30°C.

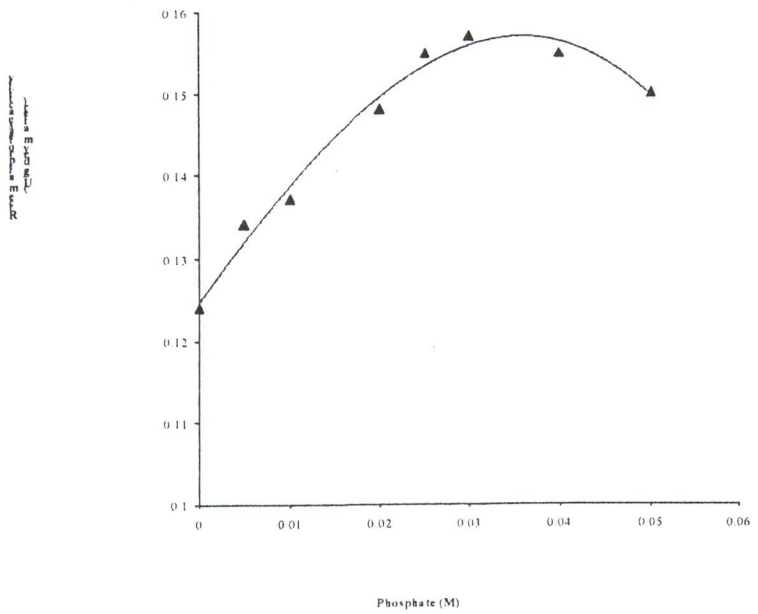

Figure 5: Effect of different concentrations of phosphate ions in phosphate buffer (pH 7.5) on the extraction of rice malt proteases.

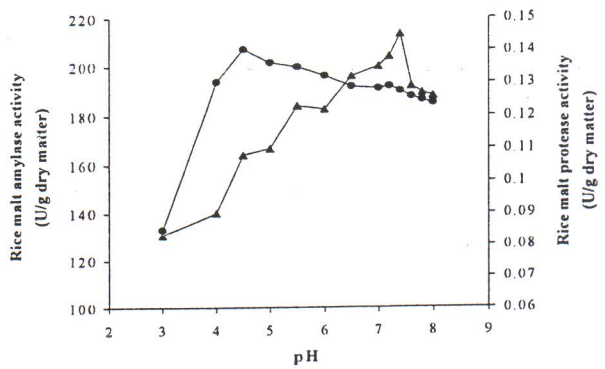

Figure 6: Effect of pH on simultaneous extraction of malt amylases and proteases from malted rice powder at 30°C for 30min with occasional shaking. (●), Rice malt amylase activity and (▲), Rice malt protease activity.

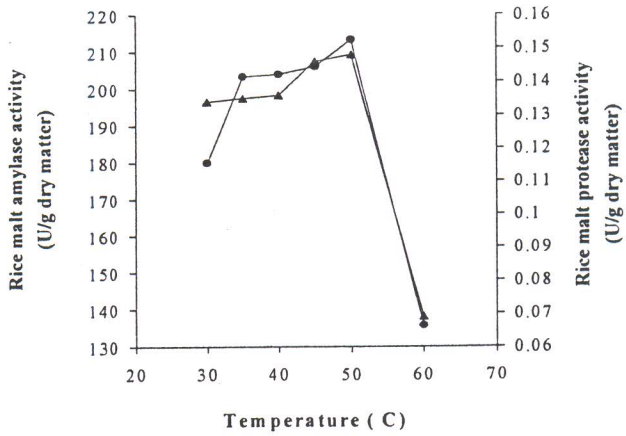

Figure 7: Effect of temperature on the simultaneous extraction of malt amylases and proteases from malted rice powder at pH 7.4 with occasional shaking for 30 minutes. (●), Rice malt amylase activity and (▲), Rice malt protease activity.

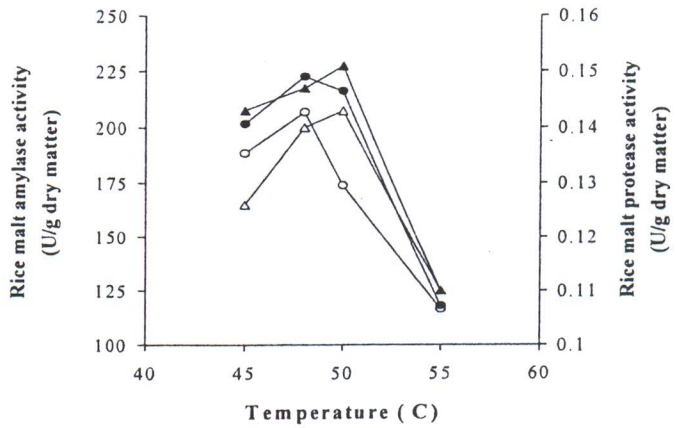

Figure 8: Combined effect of temperature and detergent (Triton X-100) on the simultaneous extraction of malt amylases and proteases from malted rice powder at pH 7.4 with occasional shaking for 30 minutes. (●), Rice malt amylase activity with detergent; (○), Rice malt amylase activity without detergent; (▲), Rice malt protease activity with detergent and (Δ), Rice malt protease activity without detergent.

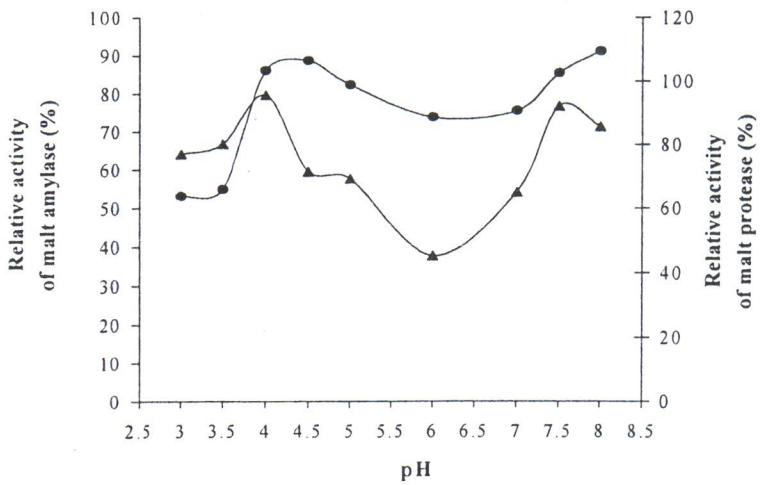

Figure 9: Effect of pH on the storage stability of malt amylases and proteases at 30°C. (●), Amylolytic activity and (▲), Proteolytic activity.

Table 1: Repeated extraction of rice malt amylases and proteases from malted rice powder.

Extraction (Number)	Amylase activity (%)	Protease activity (%)
1st	60.0	63.2
2nd	19.5	25.2
3rd	9.6	9.0
4th	6.4	2.7
5th	2.4	-
6th	2.0	-

Table 2: Comparison of amylolytic and proteolytic activities and soluble proteins contents of malted rice suspension/ supernatant in the presence and absence of Triton X-100 for repeated extraction. Malted rice powder (4th day sample) was extracted with 10.0mL portions of 0.01M phosphate buffer (pH 7.4) with and without Triton X-100 at 50°C for 30 minutes. Soluble protein content and malt enzyme activities were estimated in the initial suspension and supernatants obtained from the extraction. This procedure was carried out repeatedly for 5 times.

Suspension or Supernatant	Presence of Triton X-100			Absence of Triton X-100		
	Soluble protein (mg/g DM)	Amylolytic activity (U/g DM)	Proteolytic activity (U/g DM)	Soluble protein (mg/g DM)	Amylolytic activity (U/g DM)	Proteolytic activity (U/g DM)
Suspension	92.380	285.51	0.1892	79.450	274.74	0.1801
Supernatant 1	4.864	228.41	0.1570	4.486	199.87	0.1386
Supernatant 2	4.105	31.98	0.0259	4.014	25.88	0.0254
Supernatant 3	3.328	10.39	0.0035	3.233	08.68	0.0076
Supernatant 4	3.185	08.19	0.0024	2.726	04.37	0.0030
Supernatant 5	2.061	04.85	0	1.979	02.86	0.0019

DM – Dry matter