



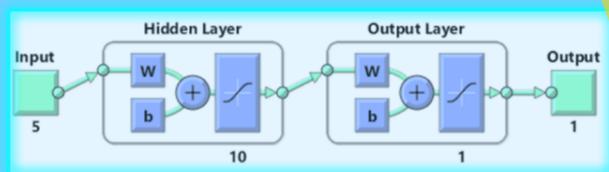
INTRODUCTION

- A recent shift in the human consumption pattern towards more sustainable and health-conscious diet has increased the demand for plant proteins globally.
- Most plant proteins are unable to compete with animal proteins in terms of utilization and consumer acceptance due to their lower functional properties.
- To overcome the limitations, conjugation of plant proteins with carbohydrates has piqued interest in recent times [1,2]



- The process of establishing or designing experiments can be difficult and time-consuming. In this study, as an example of the implementation of neural networks, amaranth plant protein, and seaweed polysaccharides were selected due to the limited availability of studies related to the conjugation of these components.
- To establish a range of parameters and create the design of experiments, we created an artificial neural network to predict the range of different parameters based on the highest conjugation efficiency of protein and a polysaccharide.

RESULTS



- 5 inputs consisting of ultrasonication power, time, temperature, the ratio of protein to sugar, and conjugation degree while the predicted output was a single row of data combining the parameters with the most efficient conjugation degree (%).
- Predicted output (providing >40% conjugation) was the parameter range consisting of 20% ultrasonication (US) power for 60 minutes at ultrasonication temperature of 75°C, and protein: polysaccharide ratio 1:1.
- This range was used to establish DOE with US power (15/20/25%), US time (30, 45, 60 minutes), temperature (45, 60, 75 °C) and ratio (1:1/1:2/2:1).
- As per prediction, the protein: polysaccharide of 1:1 and 2:1 displayed better conjugation, including the changes in the amide regions of the protein. Lower conjugation degree for 1:2 samples can be result of shielding-effect of proteins reaction terminals due to higher polysaccharide content.
- The increase in conjugation was attributed to higher cavitation and disruption phenomenon during the treatment of samples leading to an improved rate of reaction and availability of the conjugation sites on the protein and polysaccharide [3].
- Based on FTIR analysis, the amide-I region (1600-1800 cm⁻¹), compared to the native amaranth protein and the seaweed, showed a marked decrease in the peak intensity for all the samples with marked decrease in the range predicted by the neural network.
- This change can be attributed to the condensation reaction between ε-amino acids and reducing sugars signifying the initiation of Maillard reaction [4]

CONCLUSION

Our study demonstrated that, with proper training, the neural networks could be an efficient tool for predicting the range of parameters for initiation of DOE for conjugation. Furthermore, this method can be incorporated for prediction of parameters for different studies apart from conjugation.

RESEARCH APPROACH

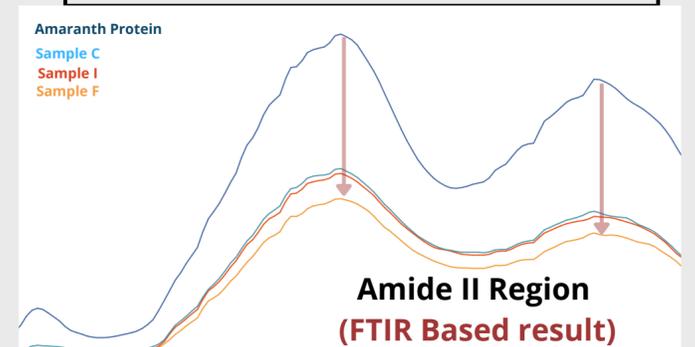
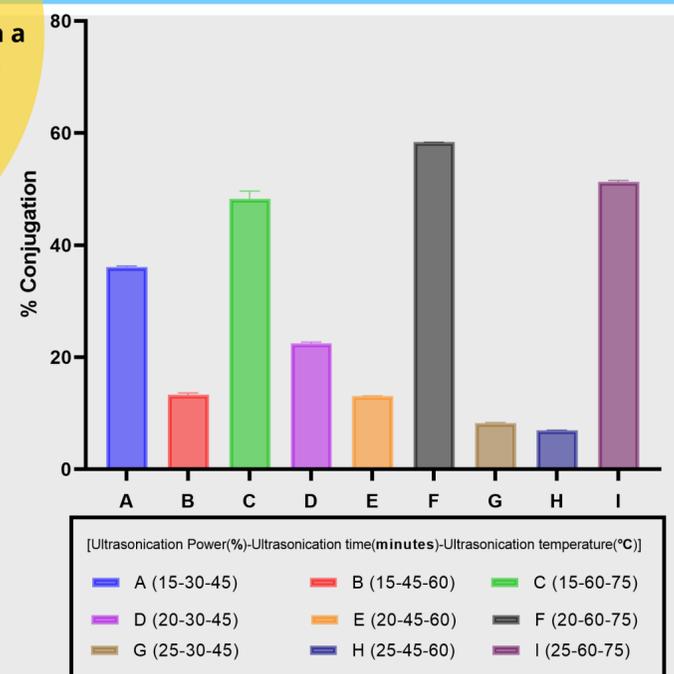
- To design the experimental conditions for this study, published data available online related to ultrasonication based Maillard reaction was collected and used for training the network.
- Using MATLAB R2020A, a feed-forward, backward propagation network was established and trained using the 'TRAINLM' function and 'LEARNGDM' adaptation learning function.
- Two layers were selected (hidden (10 neurons) and output layer) with the 'TANSIG' transfer function while the whole consolidated data consisting of the input, testing, and target data was divided into 75:25:25.
- The predicted parameters were used to establish the experiment design and validated based on actual experiments with emphasis on conjugation degree using TNBS method and changes in protein structures using Fourier Transform Infrared (FTIR) analysis.
- The degree of conjugation was then estimated using the formula, where A_o and A_t are the absorbance (dimensionless) of the sample before and after the treatment.

What are Neural Networks?

A series of algorithms that can recognize different patterns & relationships in a set of data through a process that mimics how our brain works.



$$\text{Degree of Conjugation (\%)} = ((A_o - A_t) / A_o) * 100$$



- Significant drop in the peak intensity of the sample with predicted parameters in amide-II (1470-1570 cm⁻¹) and III (1250-1350 cm⁻¹) (C-N stretching and N-H bending) regions, indicative of the reaction that resulted in consumption of amino acids

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