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Formulation and Fuzzy Modeling of Viscosity of an Orange-Flavored Carboxymethylcellulose-Whey Protein Isolate Beverage

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Formulation and Fuzzy Modeling of Viscosity of an Orange-Flavored Carboxymethylcellulose-Whey Protein Isolate Beverage*

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Abstract

The aim of this study was to employ whey proteins isolate (WPI) and carboxymethylcellulose (CMC) as stabilizers in beverage production and to evaluate viscosity of the products. Three beverages were formulated using 6% WPI combined with three different concentrations of CMC (0.1, 0.5, and 1%). The combination of 6% WPI/1.0% CMC was selected and added to three different ratios of pure orange juice (50:50 "T1," 40:60 "T2," and 15:85 "T3"). The apparent viscosity decreased as shear rate and temperature increased. Additionally, the apparent viscosity for the same treatment at certain shear rate/same temperature increased after storage. In addition, an adaptive neuro-fuzzy inference system (ANFIS) was used to model and identify the viscosity of the resulted beverages. Experimental validation runs were conducted to compare the measured values and the predicted ones. ANFIS models achieved an average prediction error of viscosity of only 9%. It is believed that this approach can be applied to predict many other parameters and properties in beverage industry.

KEYWORDS: beverage formulations, whey protein isolate (WPI), carboxymethylcellulose (CMC), apparent viscosity, fuzzy modeling

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1. INTRODUCTION

The dairy industry produces large amounts of whey as a by-product of the cheese industry. Most of whey protein is lost in the drainage without any benefit (Morr and Foegeding, 1990). The environmental pollution and high costs of disposing whey encouraged scientists to explore methods to utilize it in the food industry. Also, the high nutritional value (Damodoran, 1996) and low cost of whey make it an excellent food component (Dickinson, 1997).

Several types of proteins are used as emulsifiers in foods since they have a high proportion of non-polar groups and surface active properties (Damodoran, 1996). Whey protein is one of the most important proteins that have the capability to stabilize emulsions (Dickinson, 1997; McClements, 1999). Whey proteins are the soluble proteins in the milk after the precipitations of the caseins at pH 4.6 and 20 °C (Dallgleish, 1996). Whey proteins form about 20% of total milk proteins and are composed mainly of β -lactoglobulin (~66%) and α -lactalbumin (~13%) (Kinsella, 1984). The ability of whey proteins to form stable emulsions depends on several factors including pH, and temperature (Kinsella, 1984; Dallgleish, 1996; Singh and Ye, 2000). Because of their ability to adsorb at the oil-water interface and their good solubility, whey proteins are considered good stabilizer (Girard et al., 2002). A study investigated the effect of using other types of protein in beverage production such as using soy protein. It was reported that the addition of soy protein imparted significant beverage stability with a good nutritional value. Nonetheless, this system experienced a slight degree of bitterness (Beverage Marketing Corporation of New York, 2005).

The gums are polysaccharides classified according to their origin (Igoe, 1982). The hydrocolloids (gums) have the ability to control both the rheology and texture throughout the stabilization of emulsions, suspensions, foams and starch gelatinization (Rosell et al., 2001). Carboxymethylcellulose (CMC) is anionic polysaccharides that come from cellulose. It has a pKa value of about 4.0 and can solubilise whey at an acidic condition by acidification through the formation of insoluble complex (Hidlago and Hansen, 1969; Igoe, 1982). This polysaccharide is widely used in the beverage industry because it is inexpensive and has the ability to form complexes with whey proteins because of its anionic properties. Interaction between CMC and whey proteins located at the droplet surface of the emulsions influences the creaming behavior of the emulsions and stabilizes the emulsion through adsorption of the secondary layer of the CMC (Dickinson, 1998). Some studies investigated the emulsifying properties of WPI/CMC complex (Girard et al., 2002) in a pure system but not in formulating a new beverage product.

Fuzzy logic and fuzzy inference system (FIS) is an effective technique for the identification and modeling of complex nonlinear systems. Fuzzy logic is

particularly attractive due to its ability to solve problems in the absence of accurate mathematical models (Zadeh, 1965; Zadeh, 1973; Kasabov, 1998). The predictions of properties of the resulted beverage viscosity could be considered as a complex system, so using the conventional technology to model these property results in significant discrepancies between simulation results and experimental data. Thus, this complex nonlinear system fits within the realm of neuro-fuzzy techniques (Jang and Sun, 1995; Kosko, 1992; Yamaguchi et al, 1991).

Modeling and identification of food properties and processing has been the subject of many researchers in the food engineering field. (Perrot et al., 2003) presented a hybrid approach based on fuzzy logic and genetic algorithms to control a crossflow microfiltration pilot plant. The results of simulations and pilot tests showed that it becomes possible to impose dynamics to the process that leads to maintain the state variable at a given reference. Tsourveloudis and Kiralakis (2002) applied a rotary drying process to olive stones. They described and modeled the process using fuzzy and neuro-fuzzy techniques based on available expertise and knowledge for a given, industrial size, rotary dryer. They also used ANFIS controller based on data taken from an empirical model of the dryer under study. Samhouri, Abu Ghoush and Herald (2007) found that the neuro-fuzzy modeling technique (i.e., ANFIS) can be used to achieve very satisfactory prediction accuracy (about 98%) in a model color mayonnaise system. Also, very satisfactory prediction accuracy (about 96%) was achieved by applying neuro-fuzzy modeling technique (i.e., ANFIS) in predicting the emulsion stability and viscosity of a gum- protein emulsifier in a model mayonnaise system (Abu Ghoush, Samhouri, Al Holy and Herald, 2008).

The main motivation behind this work was to utilize the functional and dietary benefits of whey in making nutritional and good quality beverage. Both, processors and consumers have demanded the use of disposable whey in such products (Damianou et al., 2006). Therefore, the aims of this research were to take the advantage of the WPI/CMC interaction, formulate a beverage with certain properties, evaluate the beverage viscosity and construct a prediction model for the beverages viscosity using fuzzy modeling that can be used as a tool by the food processors to produce a high quality beverage product.

2. MATERIALS AND METHODS

2.1 Proteins, Polysaccharides

Whey protein isolate (WPI) was obtained from Aria food ingredients (Amba-Denmark, LACPRODAN[®] DI-9224, Denamark) and carboxymethylcellulose (CMC) was obtained from TIC-Gums (Belcamp, MD, USA).

2.2 Stages of the Study

Experimental design of this research was divided into two main stages:

2.2.1 Formulation and Evaluation of Emulsions

Preparation of Emulsions

Three solution combinations formulated with 6%WPI (preliminary study showed that it exhibited the highest solubility performance) combined with three different concentrations of CMC (0.1, 0.5, and 1%) and replicated three times. A total of 9 batches of solutions were produced in random order. Each batch was approximately 1 L. Solution combinations were immediately bottled, capped, and placed into refrigerated storage after production. These combinations were rehydrated in deionized water for 1 h and stored at 4 °C for 24 h. All solutions were adjusted to pH 4.8. The whey protein isolate/CMC complexes were prepared according to the process patented by Chen et al. (1989).

Emulsions Evaluations

Emulsion activity index (EAI), and emulsion Stability index (ESI) for all the above combinations were determined by using a turbidimetric method developed by (Pearce and Kinsella, 1978). The highest EAI and ESI from these combinations were selected for further beverage formulations. This test was used to evaluate the stability and the degree of interaction between the CMC and whey protein isolate at selected concentrations.

2.2.2 Formulation and Evaluation of Beverage

Beverage Formulations

Beverage formulations (Table 1) were developed using laboratory-scale trials. Production of the beverage was performed according to the processing procedure developed during laboratory-scale trials (Figure 1). Three beverage treatments formulated with 6%WPI, and 1.0% CMC. This combination exhibited the highest EAI and ESI. WPI-CMC was added to three different ratios of pure orange Juice (“T1” 50:50, “T2”, 40: 60, and “T3” 15: 85). The orange juice without 6%WPI/1.0% CMC served as a control. The experiments were replicated three times. A total of 12 batches of beverages were produced in random order, each batch was approximately 1L in size. Beverages were immediately bottled, capped, and placed into refrigerated storage at 4 °C after production. The beverage with

15: 85 was excluded from the study since a preliminary sensory evaluation indicated that the content of this product precipitated during storage.

Prehydration of WPI and CMC in a small portion of water, with good agitation combined with heat treatment at 80 °C for 30 s resulted in complete mixing without lumping in a relatively short period of time. This temperature was used to minimize denaturation of the heat-sensitive whey proteins. Themlij et al. (2004) reported that the whey protein denaturation was delayed until a temperature of 87 °C.

Chemical and Microbiological Properties:

CMC/WPI beverages were stored immediately after production for 8 weeks at 5 °C. Product pH was measured using a pH meter (model 744, CH-9101 Herisau, Switzerland). Triplicate measurements were taken for all samples. Total aerobic plate count was determined according to standard pour plate method (Speck, 1979) on plate count agar (Standard Plate Count Agar, Conda Laboratories, S.A., Spain) incubated at 37 °C for 48 h. Coliform count was determined using Violet Red Bile Agar and Eosin Methylene blue agar (Conda Laboratories, S.A., Spain) incubated at 37 °C for 24 h, respectively.

Rheological Properties:

Rheological measurements of the developed beverage samples were determined using a Brookfield viscometer (Model LVDV-E, Brookfield Laboratories, Middlebooro, MA, USA). The equipment operates at a rotor speed range 0.3-200 rpm. Shear rate equation was obtained from manufacturer's manual as follows:

$$\dot{\gamma} = 0.22N \dots\dots\dots(\text{equation 1})$$

Where $\dot{\gamma}$: is shear rate in 1/s.

N: is viscometer spindle rotation speed (rpm).

The viscometer can be used to construct rheograms by providing apparent viscosity and shear stress data. A thermostatically controlled water bath was used to maintain the temperature of the beverage constant. Rheological measurements were conducted at 8 °C and 24 °C (representing refrigeration and room temperatures) at the following rpm values: 100, 60, 50, 30, 20, 12, 10, and 6. The measurements were taken immediately after beverage preparation and repeated after 8 weeks of storage. Prior to measurements, samples were heated in a water bath to reach steady state temperatures of 8 °C and 24 °C to resemble the temperatures as the product is consumed. All measurements were obtained in triplicate.

2.3 Statistical Analysis

A two-way factorial classification in complete randomized design (CRD) was used. Data were analyzed using statistical analysis software (version 8.2, SAS Institute Inc., Cary, NC). Three batches of beverage were produced for each treatment. Analysis of variance (ANOVA) and means separations were calculated by the general linear model procedure (Proc GLM). Comparisons among treatments were analyzed using Fisher Least Significant Difference (LSD). Treatment means were considered significant at $P < 0.05$.

2.4 Fuzzy Modeling of Output Properties

Neuro-fuzzy is an associative memory system that consists of fuzzy nodes instead of simple input and output nodes. Neuro-fuzzy uses neural network learning functions to refine each part of the fuzzy knowledge separately. Learning in a separated network is faster than learning in a whole network. One approach to the derivation of a fuzzy rule base is to use the self learning features of artificial neural networks, to define the membership function based on input-output data. A fuzzy inference system (consisting of rules, fuzzy set membership functions, and the defuzzification strategy) are mapped onto a neural network-like architecture.

Adaptive neuro-fuzzy inference system (ANFIS) is a fuzzy inference system implemented in the framework of an adaptive neural network. By using a hybrid learning procedure, ANFIS can construct an input-output mapping based on both human-knowledge as fuzzy. If-Then rules and stipulated input-output data pairs for neural networks training. ANFIS architecture is shown in Figure 2, where x and y are the inputs, f is the output, A_i and A_n^2 are the input membership functions, w_i and w_n^2 are the rules firing strengths. Five network layers are used by ANFIS to perform the following fuzzy inference steps: (i) input fuzzification, (ii) fuzzy set database construction, (iii) fuzzy rule base construction (iv) decision making, and (v) output defuzzification. This is a multi-layered neural network architecture where the first layer represents the antecedent fuzzy sets, while the consequent fuzzy sets are represented by the middle layers, and the defuzzification strategy by the output layer. The nodes which have 'square' shape are those containing adaptable parameters, whereas the 'circular' nodes are those with fixed parameters.

ANFIS is more powerful than the simple fuzzy logic algorithm and neural networks, since it provides a method for fuzzy modeling to learn information about the data set, in order to compute the membership function parameters that best allow the associated fuzzy inference system to track the given input/output data (Jang, 1993). ANFIS also employs sugeno-type fuzzy inference system, which is a natural and efficient modeling tool, and is suited for modeling non-

linear system by interpolating between multiple linear models. In addition, ANFIS is more powerful than neural network system since it is better than all of them in convergence rates (running time), average training error, root mean square error, and the coefficient of correlation, and it has a built-in ability to validate the modeled system. On the other hand, ANFIS is much more complex than the fuzzy inference systems, and is not available for all of the fuzzy inference system options. It only has a single output, and no rule sharing. In addition, ANFIS cannot accept all the customization options that basic fuzzy inference allows. That is, no possibility to make our own membership functions and defuzzification functions; the ones provided by ANFIS must be used.

ANFIS Modeling of Viscosity

An adaptive neuro-fuzzy inference system (ANFIS) is an architecture which is functionally equivalent to a Sugeno-type fuzzy rule base. ANFIS is a method for tuning an existing rule base with a learning algorithm based on a collection of training data. This allows the rule base to adapt. Training data is used to teach the neuro-fuzzy system by adapting its parameters (which in essence are fuzzy set membership function parameters) and using a standard neural network algorithm which utilizes a gradient search, such that the mean square output error is minimized.

The architecture of ANFIS, illustrated in Figure 2, has five layers to accomplish the tuning process of the fuzzy modeling system. The five layers are:

- 1) Layer 1: Every node in this layer is an adaptive node with a node function (i.e., membership function). Parameters of membership functions are referred to as premise or antecedent parameters.
- 2) Layer 2: Every node in this layer is a fixed node, which multiplies the incoming signals and sends the product out. Each node represents the firing strength of a fuzzy rule.
- 3) Layer 3: Every node in this layer is a fixed node which calculates the ratio of the one firing strength to the sum of all rules' firing strengths. The outputs of this layer are called normalized firing strengths.
- 4) Layer 4: Every node in this layer is an adaptive node with a node function (i.e., linear combination of input variables). Parameters in this layer are referred to as consequent parameters.
- 5) From the ANFIS architecture Layer 5: The single node in this layer is a fixed node that computes the overall output as the summation of all incoming signals, shown in Figure 2, it is observed that given the values of premise parameters, the overall output can be expressed as a linear combination of the consequent parameters.

ANFIS applies two techniques in updating parameters. For premise

parameters that define membership functions, ANFIS employs gradient descent back-propagation neural networks to fine-tune them. For consequent parameters that define the coefficient of each output equation, ANFIS uses that least squares method to identify them. This approach is called the hybrid learning method. More specifically, in the forward pass of the hybrid learning method, functional signals go forward until layer 4 and the consequent parameters are identified by the least square estimate. In the backward pass, the error rates propagate backward and the premise parameters are updated by the gradient descent.

ANFIS modeling and prediction of the viscosity output starts by obtaining a data set (input-output data points) and dividing it into training and validating data sets. Each input/output pair contains four inputs (i.e., time, shear rate, temperature, and treatment) and one output (i.e., viscosity). The training data set is used to find the initial premise parameters for the membership functions by equally spacing each of the membership functions. A threshold value for error between the actual and desired output is determined. The consequent parameters are computed using the least squares method. Then, an error for each data pairs is found. If this error is larger than the threshold value, the premise parameters are updated using the back propagation neural networks. This process is terminated when the error becomes less than the threshold value. Then, the testing data points are used to compare the model with actual system for validating purposes.

3. RESULTS AND DISCUSSION

3.1 Solution Combinations Evaluation

The EAI of 6%WPI significantly increased with the addition of 0.1, 0.5, and 1 % CMC by 50, 64, and 84 %, respectively (Figure 3). The ESI of 6%WPI significantly increased with the addition of 0.1, 0.5, and 1% CMC by 50, 77, and 97 %, respectively (Figure 4). It is clear that as CMC concentrations increases the stability of beverage. This could be due to the formation of a protein-polysaccharides network. The interaction between CMC and whey proteins located at the droplet surface of the emulsions influences the behavior of the solutions and stabilizes the emulsion through adsorption of the secondary layer of the CMC, thus increasing ESI. WPI improves the surface properties of food systems by forming a protective steric barrier around insoluble droplets. At the same time, CMC improves the steric stabilizing properties by forming a thick secondary layer on the outer side of protein. These results are confirmed with the results obtained by others (Dickinson 1998; Rosell et al. 2001; Giraard et al. 2002; and Damianou et al. 2006).

3.2 Formulations and Evaluation of Beverages

Chemical and Microbiological Properties

The pH of beverage remained stable for all formulations during the 8-week refrigerated storage period. Beverages pH values were 4.70, and 4.63 for T1, and T2, respectively.

Despite the high water amounts, and nutrients contents, the low pH of the product prevented growth of microorganisms. Beverages aerobic plat count (APC/ml) were 30, and 47 for T1, and T2 treatments, respectively and remained stable for all formulations during the 8-week refrigerated storage period. This low count is probably spore forming bacteria that does not have the capability to germinate under the acidic conditions of the beverage. The coliform count was undetectable (<10/ml) for T1, and T2 treatments. This is most likely because of relatively high pasteurization temperature (80 °C for 30s) and low pH (~ 4.70) that impede the growth of coliform.

Rheological Properties

A power law model that is widely used in theoretical analysis of engineering calculations was used to explain the relationship between shear stress and shear rate as follows:

$$\tau = K\gamma^n \dots\dots\dots(\text{equation 2})$$

Where τ : is the shear stress (Pa), K is consistency coefficient, n is the flow behavior index.

For a power law model, the apparent viscosity can be defined as follows:

$$\mu_a = K\gamma^{n-1} \dots\dots\dots(\text{equation 3})$$

Where μ_a : is the apparent viscosity in Pa.s.

A plot of apparent viscosity against shear rate_(a gradient of velocity in a flowing material. The SI unit of measurement for shear rate is sec^{-1}) was obtained for each treatment. The plot was fitted to a power function where K and n were obtained directly from the power law function. The results for n, K, and R^2 are shown in Table 2. The results showed that n values were less than one, indicating a shear thinning behavior of the beverage samples. For sample T1, the results showed that n decreased from 0.652 to 0.620 as temperature increased from 8 to 24 °C, and K decreased from 0.077 to 0.058 for the same temperature increment.

This decrease was due to the decrease in apparent viscosity as temperature increased. The sample T2 showed a similar trend with n decreasing from 0.392 to 0.328 and K decreasing from 0.11 to 0.081 as temperature increased from 8 to 24°C. These values reflect the nature of shear-thinning fluids.

Equivalent results for measurement taken after 8 weeks are shown in Table 3. Flow behaviour index decreased after 8 weeks. The relationship between apparent viscosity and shear rate for formulation T1 and T2 at different temperatures is shown in Figure 5. The reduction in apparent viscosity with increasing shear rate is most probably due to the structural breakdown of the formulations by hydrodynamic forces generated and the increased alignment of the constituent molecules, since shearing causes progressive deformation and disruption of droplets resulting in less resistance to flow (Alparslan and Hayta, 2002; Rao, 1999). Obviously, the viscosity increased significantly for all temperatures and formulations after 8 weeks and the relationship between apparent viscosity and shear rate became less dependent on temperature and formulation type. This indicates that viscous behavior of both formulations became more stable after 8 weeks probably due to development of cross polymerization networks (Figure 6) (Girard et al. 2002; and Damianou et al. 2006).

ANFIS Modeling

The fuzzy logic toolbox of Matlab 7.0 was used to obtain the results, and to build a fuzzy model for the viscosity. Figure 7 shows the training curve for building a fuzzy model for viscosity. 160 data points were used for training the system to predict the viscosity. 1500 neural nets learning epochs were used to get a low error of training (i.e., RMSE = 7.48 or 3 percent of the training data range = $Maximum - Minimum = 240$). A comparison between the actual and ANFIS predicted viscosity after training is shown in Figure 8, which shows that the system is well-trained to model the actual viscosity.

Twenty five data points, which are different and independent from the training data, were used to validate the system. The final fuzzy inference system that predicts the viscosity is shown in Figure 9. As illustrated in Figure 9, a two (Gaussian) type membership functions for each input (4 inputs) resulted in high accurate prediction results.

Models Validation

The ANFIS prediction model for viscosity was validated by selecting a certain number of data points (i.e., 25 points), different from the other 160 points used for ANFIS training. Each validation data point (i.e., time, shear rate, temperature, and

treatment) was fed into the system, and then the predicted properties (i.e., viscosity) were compared to the actual values of the measured viscosity. The average percent errors in the modeling of viscosity was 9% achieving an accuracy of viscosity prediction of 91%. Table 4 shows the data points used in system's validation along with the actual and predicted viscosity values, and the percent errors in the predictions. This table shows that the ANFIS predicted values are a close match of the actual ones.

4. CONCLUSIONS

High quality and shelf stable beverages were formulated with 6% WPI/1% CMC and pure orange juice combined at 50:50 and 40:60 ratios, respectively. The apparent viscosity decreased as shear rate and temperature increased. Additionally, the apparent viscosity for the same treatment at certain shear rate/same temperature increased after storage. ANFIS models achieved an average prediction error of viscosity of only 9%. The present study shows that ANFIS is a technique that can be used efficiently to predict the food properties. It is believed that this approach can be applied to predict many other parameters and properties in food industry.

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