

# Functional properties of goat cheese protein hydrolysed. Evaluation by artificial neural network

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## Abstract

*The aim of this work is to study key functional parameters of the goat cheese protein hydrolysates. A Plackett-Burman Statistical Design, Response Surface Methodologies and Artificial Neural Network are applied to describe the effects of different factors (pH, Temperature, Time of hydrolysis, Amount of added buffer and Enzyme : Substrate ratio) on the following functional parameters of goat cheese proteins, which are hydrolysed by papain: Free Amine Nitrogen (NA), Total Soluble Nitrogen (NT), Solubility (PSI), Water Holding Capacity (WHC), Emulsifying Activity Index (EAI), Emulsifying Stability Index (ESI), Viscosity ( $\eta$ ), Held Water (HW), Surface Hydrophobicity (So), Foaming Capacity (FC) and Foam Stability (FS). According to our results, the release of soluble proteins from goat cheese to the supernatant (NT) and the hydrolysis degree of proteins into the supernatant (NA) increased until 443% and 273%, respectively. PSI, WHC, EAI, ESI and HW increased until 443%, 159%, 0.88%, 324% and 64% respectively.  $\eta$  decreased until 33% and So (bitter peptides indicator) was reduced until 98.8 %, regard to the original protein isolates. FC and FS were extremely low or null. Predicted values were experimentally confirmed and compared with those of original protein isolates.*

**Keywords:** Goat Cheese, Protein Hydrolysis, Functional Properties, Plackett Burman Statistical Design, Response Surface Methodology, Artificial Neural Network.

## I. Introduction

Goat cheese is an artisan product which is gaining momentum in the world market. Cheese proteolysis has a high impact on the development of mature cheese texture, taste and aroma. However, the formation of hydrophobic peptides can cause an undesirable bitter taste [1]. *Functional properties* of cheese can be modified by enzymatic proteolysis. Cheese proteins are highly nutritive, but they may display inadequate *functional properties* when they are added to a food system.

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*Functionality* is the ability of a food ingredient to interact with other components and give *desirable* properties to a food system [2]. Data analysis with multivariate statistical techniques has been successfully used on sensory parameters after proteolysis [3], but there are no previous studies that have applied those techniques to functional properties study of hydrolysed goat cheese proteins. The aim of this work is to study of key functional parameters of the goat cheese proteins hydrolysates. A Plackett-Burman Statistical Design, Response Surface Methodologies and Artificial Neural Network are applied to describe the effects of different factors (pH, Temperature, Time of hydrolysis, Amount of added buffer and Enzyme : Substrate ratio) on the following functional parameters of goat cheese proteins, which are hydrolysed by papain: NA, NT, PSI, WHC, EAI, ESI,  $\eta$ , HW, So, FC and FS.

## II. Materials and Methods

### A. Sample Preparation

Aqueous dispersions of previously processed semi-hard goat cheese were prepared using a laboratory homogenizer during 2 min. 10 g of processed sample with different amounts of phosphate buffer (pH: 4 or 8) were stirred at 200 rpm in a rotating orbital shaker (GFL, Germany). Temperatures were 40 and 60 °C during 40 or 260 min, respectively. Commercial papain from *Carica papaya* (Fluka, 3.11 U/mg, EC 3.4.22.2) was the proteolytic enzyme used in all trials.

### B. Statistical Design of Experiments (DOE)

A two-level Plackett-Burman´ DOE was performed using the following factor ranges, which were based on previous experimental trials: pH: 4 and 8; Temperature: 40 and 60 °C; Time of hydrolysis: 40 and 260 min; Amount of added buffer: 13 and 30 g of buffer / g of protein; Enzyme: Substrate ratio (E:S):  $5.5 \cdot 10^{-3}$  and 0.2 mg of papain / g of cheese casein. According to Plackett-Burman´ DOE, 12 runs (by duplicate) were carried out, and the following responses were measured: NA, NT, PSI, WHC, EAI, ESI,  $\eta$ , HW, So, FC and FS. A daily experimental order was followed to avoid biased results caused by blocking effects. After finishing each experimental run, samples and blanks were heated to boiling point so as to stop enzymatic activity. They were cooled at -4 °C and centrifuged at 3,000 x g, during 15 min. All determinations were carried out by duplicate and they were compared with the same amount of processed goat cheese without papain (blank). Besides, 5 runs were also replied at the

design center. All analytical grade chemicals used in this work were supplied by Sigma-Aldrich Co. (St. Louis, USA).

### **C. Free Amine Nitrogen (NA) and Total Soluble Nitrogen (NT)**

NT and NA were determined by the Kjeldhal and Phenol-hypochloride methods in the free fat supernatants, using filter paper Whatman N° 1 [4, 5].

### **D. Solubility (PSI)**

PSI is the percentage of supernatant soluble protein with respect to the total protein contents, by Kjeldahl method [6].

### **E. Water Holding Capacity (WHC)**

WHC has been estimated as the amount of water that original and hydrolysed proteins can retain after the action of a centrifugal force under standardized conditions, according to [7].

### **F. Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI)**

The interface area that can be covered by a protein is related to its capacity to form and stabilize emulsions. EAI and ESI were measured by the turbidimetric method of Pearce and Kinsella (1978), modified by Tang et al. (2006) [8].

### **G. Viscosity ( $\eta$ )**

Apparent  $\eta$  of hydrolysates samples and blanks were determined by means of a programmable rheometer (Brand Brookfield, Model DV-III), according to Hermansson method (1979) [9]. 9 routine from 100 to 140 rpm, at 24 °C were performed, and the average apparent  $\eta$  was obtained.

### **H. Held Water (HW)**

HW was determined by means of a method developed by Piva et al. (1981) [7].

### **I. Surface Hydrophobicity (So)**

So of original and hydrolysed samples was performed with a probe of 1-anilino-8-naphthalene sulfonate (ANS), using Kato and Nakai's method (1980) [10]. Fluorescence intensity (FI) was measured with a fluorescence spectrophotometer (Perkin-Elmer 2000) at 365 nm (excitation) and 484 nm (emission). FI vs. Protein concentration was plotted, and the initial slope of linear regression analysis was used as an index of protein surface hydrophobicity (So).

### **J. Foaming Capacity (FC) and Foam Stability (FS)**

FC was measured by using a graduated glass column with a porous glass disc (type G 4) at the bottom. Gas N<sub>2</sub> (fr, ml/min) was insufflate at a flow of 180 ml/min through 30 ml of supernatant of hydrolysed goat cheese protein (containing 0.5 to 2 mg of protein/ml), until a final volume (Vf, ml) of 275 ml of

foam was obtained. The time taken to obtain this volume (tf, min) was registered. FS was measured as the specific constant of drainage rate (FS, ml<sup>-1</sup>.m<sup>-1</sup>) [11]. Vmax is the volume of liquid that was gotten up to the foam at the end of the bubbling period (ml). t<sub>1/2</sub>: time for drainage of the half of the entrapped liquid in the foam at the end of the bubbling (min).

$$FC = Vf / (fr \cdot tf) \quad (1)$$

$$FS = 1 / V_{max} \cdot t_{1/2} \quad (2)$$

## **K. Artificial Neural Networks (ANNs)**

Artificial neural networks (ANNs) are a family of statistical learning models inspired by biological neural networks, and are used to estimate or approximate functions that can depend on a large number of inputs and are generally unknown. ANNs are presented as systems of interconnected neurons that have numeric weights which can be tuned based on experience [12]. In this work, an ANNs back-propagation type of errors was proposed due to the complexity and multivariate characteristics of the studied system. A matrix containing 7800 simulation data generated by a design of experiments, and limited to the operating range was used to train the network. The results were interpreted by Response Surface Methodologies.

## **III. Results and Discussion**

The effect of protein hydrolysis of goat cheese on several functional properties which were evaluated by mean of a Plackett–Burman's DOE and ANNs, are described below.

### **A. Free Amine Nitrogen content (NA) and Total Soluble Nitrogen content (NT)**

Statistical analysis of NA showed average maximal values of 1.463 g/ml at highest pH, temperature and E:S ratio (Fig. 1a). This behaviour was due to the fact that papain is an active and stable enzyme up 80 °C and has optimal pH 9 [13]. Statistical analysis of NT showed average maximal values of 11.28 g/l at lowest pH and amount of added buffer, and at the highest E:S ratio and time of hydrolysis (Fig. 1b). This means that the hydrolysis degree of soluble proteins (NA and NT) was increased until 273% and 443%, respectively, regard to the original protein isolates.

### **B. Protein Solubility Index (PSI) and Viscosity ( $\eta$ )**

Statistical analysis of PSI showed average maximal values of 93.58 % at highest E:S ratio. Statistical analysis of  $\eta$  showed average maximal values of 1.69 cP at the highest pH and temperature and at the lowest amount of added buffer. These values were 443 % higher and 33% lower than those obtained in the original protein isolates, respectively.

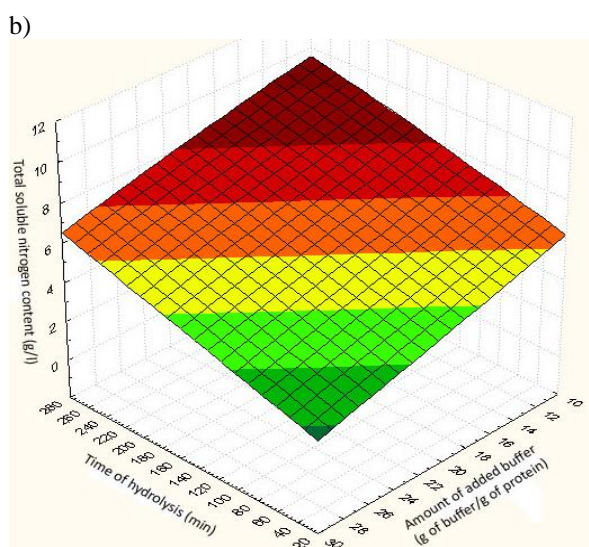
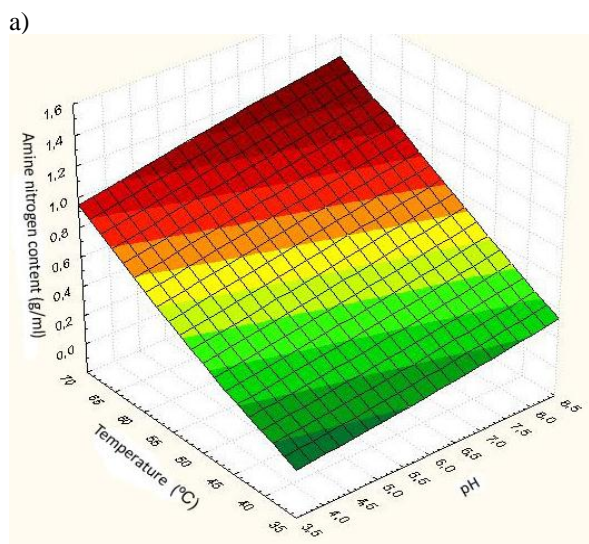


FIG. 1. A) EFFECT OF pH AND TEMPERATURE ON NA. B) EFFECT OF TIME OF HYDROLYSIS AND AMOUNT OF ADDED BUFFER ON NT, FOR HYDROLYSED GOAT CHEESE PROTEINS AFTER APPLIED ANN'S.

### C. Emulsifying properties (EAI and ESI)

Statistical analysis of EAI showed average maximal values of 1489 m<sup>2</sup>/g (at lowest pH and E:S ratio) and minimal ones of 156 m<sup>2</sup>/g. These values were 0.88 higher and 57 % lower than those obtained in the original protein isolates, respectively. Nevertheless, statistical analysis of ESI showed average maximal values of 136 min, at highest E:S ratio. This value was 324% higher than that obtained in the original protein isolates. Then, the increase of the hydrolysis degree decreases EAI but increase ESI.

### D. Foaming properties (FC and FS)

Statistical analysis of FC showed average maximal values of 1.25 at lowest pH, temperature, amount of added buffer and time of hydrolysis). FC in original protein isolates was null. Statistical analysis of FS do not showed significant values in both hydrolyzed and original protein isolates.

### E. Water Holding Capacity (WHC) and Held Water (HW)

Statistical analysis of WHC showed average maximal values of 7.55 g H<sub>2</sub>O / g pellet at the highest temperature and amount of added buffer and at the lowest pH and time of hydrolysis. Then, protein hydrolysis increases regard to the original protein isolates. Statistical analysis of HW showed average maximal values of 58% at highest temperature, pH and time of hydrolysis and at lowest E:S ratio. Then, protein hydrolysis increases WHC and HW in 159% and 64%, respectively, regard to the original protein isolates.

### F. Surface Hydrophobicity (So)

Statistical analysis of So showed average minimal values of 4.54 IF/mg of protein at the highest amount of added buffer (Fig. 2 a,b). Then, bitter peptides were reduced to 98.8 % from the original protein isolates.

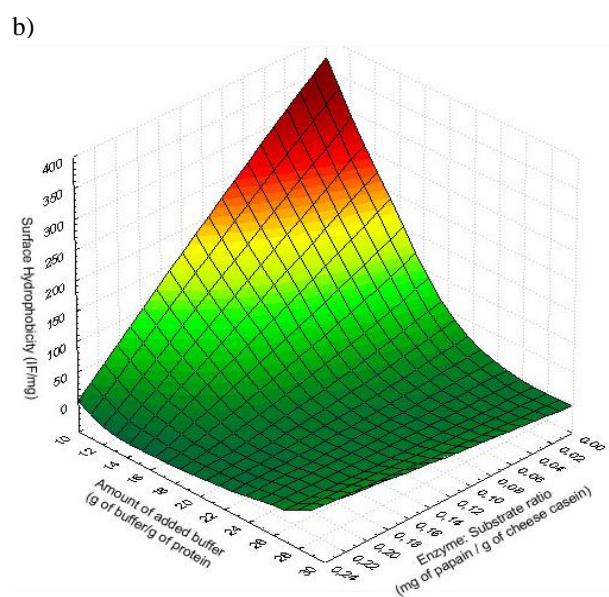
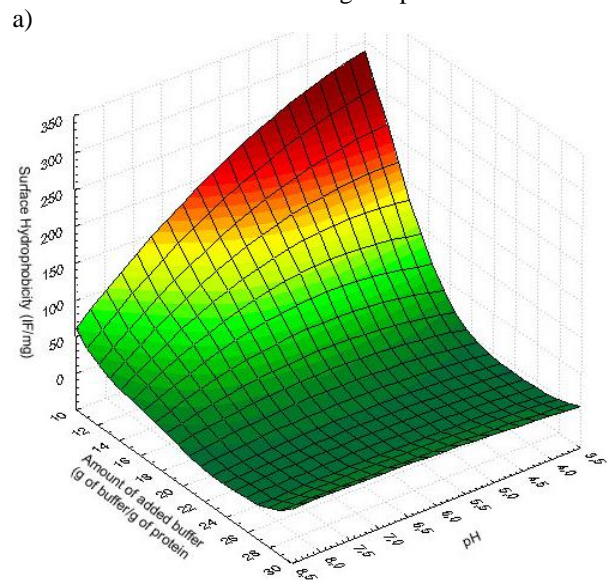


FIG. 2. A) EFFECT OF pH AND AMOUNT OF ADDED BUFFER ON So. B) EFFECT OF AMOUNT OF ADDED BUFFER AND E:S RATIO ON So, FOR HYDROLYSED GOAT CHEESE PROTEINS AFTER APPLIED ANN'S.

Table 1 summarizes the levels of significant factors that maximize the functional parameters of the goat cheese protein hydrolysates, after applying to Plackett-Burman Statistical Design and Artificial Neural Network.

TABLE 1. LEVELS OF SIGNIFICANT FACTORS THAT MAXIMIZE THE FUNCTIONAL PARAMETERS OF THE GOAT CHEESE PROTEIN HYDROLYSATES, AFTER APPLYING TO PLACKETT-BURMAN STATISTICAL DESIGN AND ARTIFICIAL NEURAL NETWORK.

↑ HIGH LEVEL. ↓ LOW LEVEL. --- NON SIGNIFICANT FACTOR.

Functional Parameter	pH	Temperature (°C)	E:S ratio (mg papain /g casein)	Amount of added buffer (g/ g protein)	Time of hydrolysis (min)
NA	↑	↑	↑	---	---
NT	↓	---	↑	↓	---
PSI	---	---	↑	---	---
η	↑	↑	---	↓	---
EAI	↓	---	↓	---	---
ESI	---	---	↑	---	---
FC	↓	↓	---	↓	↓
FS	---	---	---	---	---
WHC	↓	↑	---	↑	↓
HW	↑	↑	↓	---	↑
So	---	---	---	↑	---

## IV. Conclusions

In general, any additive based on hydrolysed protein of goat cheese requires flavoring power enhanced (NA, NT), high solubility (PSI) and absence of bitter peptides (So).

Analysis of the functional properties by ANN's allow us to conclude that those properties are achieved at highest values of pH (8), temperature (65°C), amount of added buffer (27 g / g of protein) and E: S ratio (0.2 M). Time of hydrolysis did not influence under the studied range.

Nevertheless, if high WHC and HW were required in the formulation of ingredients or additives based on goat cheese, E:S ratio, pH and time of hydrolysis should be regulated.

Besides, if high EAI and FC were required, the lowest values of pH (4), temperature (40°C), amount of added buffer (13 g / g of protein), E: S ratio ( $5.5 \cdot 10^{-3}$  M) and time of hydrolysis (40 min), would have to be selected. ANN's demonstrated to be a versatile and useful tool for predicting functional properties of protein hydrolysates and the obtained results could be optimized and scaled.

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