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Formulation and Evaluation of Corn Pancakes Containing Bovine Plasma Protein and Tender Corn

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Abstract: The purpose of this research was to fortify and evaluate some nutritional properties of a new product (corn pancakes), prepared with tender corn and bovine plasmatic protein. In doing so, two products were formulated. Both products were analyzed to determine their yield as well as their content of protein, fat, fiber, moisture, ash and essential amino acids. In addition to that, microbiological quality, degree of likeness, protein efficiency ratio and digestibility were also analyzed. All the results showed that the product containing bovine plasma had a higher yield, moisture and protein content ($p<0.05$). In almost all cases, the product containing bovine plasma met or exceeded the established ideal requirements of essential amino acids. The Protein Efficiency Ratio (PER), shows that the animals fed with the experimental diet, showed a weight gain of 2.64 g per each g of protein intake. Corn pancakes had acceptable sensory score 88.4% for taste and 95.3% for color. In conclusion, due to its acceptability and highly nutritious value, the product containing bovine plasma could be used as an alternative to help solve the nutritional problems the population is facing these days.

Keywords: Amino acid, bovine plasma, chemical composition, corn pancakes, nutritional quality

INTRODUCTION

The growth of world demand for protein-enriched products generates a great interest in the search of new protein sources of higher nutritional value and, therefore, in the use of cutting edge technologies to achieve that goal. Blood plasma protein has been extensively used because of its functional and nutritional properties (Tybor and Dill, 1975; Márquez *et al*., 1997; Viviane *et al*., 2003; Del Hoyo *et al*., 2008; Herrero *et al*., 2009; Rodríguez *et al*., 2010). Various food applications for blood plasma have been reported in the literature. Blood plasma has been incorporated into products such as cookies, pasta, meat products, protein-based films, *Lactobacillus* culture medium etc. (Barboza *et al*., 1997; Yousif *et al*., 2003; Viana *et al*., 2005; Benítez *et al*., 2008; Salgado *et al*., 2011; Rodríguez *et al*., 2011).

The main functional properties of plasma proteins are the ability to produce and stabilize foams and emulsions and the ability to form heat-induced gels (Suter *et al*., 1976; Pares *et al*., 1998; Cofrades *et al*., 2000; Pietrasik *et al*., 2007). Studies on gelling, emulsifying and foaming abilities suggest that plasma could replace some widespread ingredients, such as egg albumen and may increase their nutritional value (Raëker *et al*., 1995; Viviane *et al*., 2003; Davila *et al*., 2007; Del Hoyo *et al*., 2008).

Plasma proteins have good functional properties, thus making it easy to incorporate them into protein-deficient foods for improving their nutritional and functional properties.

Maize contains 7-13 g/100 g proteins. However, the quality of maize proteins is poor, because they are deficient in the essential amino acids lysine and tryptophan and hence should be supplemented with proteins rich in these amino acids so as to optimize the utilization of the proteins supplied in the diet (Milán-Carrillo *et al*., 2006).

Three approaches have been tried: genetic manipulation, processing and fortification (Ortega and Bates, 1983; Ayala *et al*., 2009; Gómez-Galera *et al*., 2010; Naqvi *et al*., 2011). There have been many efforts to fortify maize, with outstanding results, but unfortunately fortification has not been implemented to a large extend (Ortega and Bates, 1983).

In Venezuela corn is used to prepare arepa (Cuevas *et al*., 1985) and corn pancakes. The introduction of new food products with organoleptic characteristics similar to

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the traditional ones, would contribute to better and faster consumer acceptability; so every technique, aimed to improve and increase the nutritious value of any particular food, intended for human consumption, represents a challenge. Therefore, the purpose of this research was to fortify and evaluate a new product (corn pancakes), prepared with tender corn and bovine plasmatic protein, which could be used as food alternative for the population.

MATERIALS AND METHODS

Blood samples and separation of plasma proteins: Blood was obtained from an industrial slaughter house using sterile bleeding containers containing sodium citrate as anticoagulant (2% w/v final concentration). The blood was immediately taken to the laboratory, where it was centrifuge (INTERNATIONAL™ K Nº 69984M23) at 2500 x g for 30 min to separate the red cells and plasma. Plasma was kept at 4ºC until use.

Raw material: The tender corn (*Zea mays* L.) was obtained from a local supermarket. It was then peeled and the kernels were separated from the cob using a stainless steel knife and kept in sealed plastic containers.

Experimental design and formulation: The research was done in two phases. The first one helped determine the feasibility of using bovine plasmatic protein as a fortifying ingredient in the preparation of corn pancakes. In doing so, several formulas were attempted in order to select one, which would allow the use of the greatest amount of plasma without affecting the mixture, when being processed to obtain the final product. During this research, a total of 6 formulas were presented namely A, B, C, D, E and F. Formula A (control, without plasma) was made with 53.5% tender corn and 40% water; formula B contained 65.5% tender corn and 30% plasma; formula C had 58.5% tender corn and 35% plasma; formula D was made with 53.5% tender corn and 40% plasma; formula E contained 48.5% tender corn and 45% plasma; finally, formula F was made with 43.5% tender corn and 50% plasma. The rest of the ingredients, sugar (6%) and salt 0.5%), were added in equal proportions to all formulas.

Table 1: Ingredients (g/100)

	Formulation	
Ingredients	A (%)*	D (%)*
Tender corn	53.5	53.5
Water	40	-
Plasma	-	40
Sugar	6	6
Salt	0.5	0.5

* (A: Control; D: Product formulated with plasmatic protein)

During the second phase, a total of 216 samples (108 without plasma and 108 with plasma) were prepared during a period of 3 months, at a rate of 36 "corn pancakes" every 15 days (6 batches). The D formula was selected out of all formulas. The ingredients used to prepare formulas A (control) and D are shown on Table 1. The kernels were separated from the cob and processed for one minute in a blender (ELECTROMASTER™). Then the rest of the ingredients were added and processed for another minute. Following that, the mixture was divided into portions of 100 g and cooked for 1 min then turned over and cooked for another minute, using a Teflon® frying pan. To complete the cooking phase, the corn pancakes were turned over again to the original side. The corn pancakes were packed in individual plastic bags and stored in a refrigerator at 4ºC for 1, 2, 3, 4 and 5 days.

Cook yield: Cooking yield of corn pancakes was determined by measuring the weight of six units for each batch and calculating weight differences for corn pancakes before and after cooking according to the following equation:

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight}}{\text{Raw weight}} \times 100$$

Chemical analysis: A total of 6 units (corn pancakes) were selected randomly from each batch. They were homogenized using a food processor (OSTER™) and kept refrigerated at a temperature of 5ºC until they were analyzed. The content of proteins, fat, crude fiber, ashes and humidity of the final product were determined following the method established by (AOAC, 2004). The metabolic energy was determined using the empirical method proposed by Livesey (1995).

Amino acid analysis: Amino acids were analyzed by high performance liquid chromatography. A Shimadzu model LCC-6A HPLC, equipped with a FLD-6A fluorescence detector, two LC 6A pumps, a SCL-6B auto injector, CTO-6A column oven and C-R4A chromatopack integrator was used throughout the experiments.

An altex ultrasphere ODS, C-18, 15 cm length x 4 mm ID, 5 um column was used. Two solvent systems were used. Solvent A was composed of acetate buffer (0.05 M), methanol and tetrahydrofurane (80:19:1). Solvent B was composed of methanol and acetate buffer (80:20). A Sigma laboratory standard solution 50 µmol/mL amino acid concentrations was used as a reference.

A precolumn derivatization of the amino acids was performed. Samples of 0.02 mL were injected into the column. Flow rate was 1 mL/min. Fluorescence was read at 470 nm with an excitation wave length of 350 nm. Peak areas were used for quantitative calculations.

Biological tests: The protein quality of the product, made with tender corn and bovine plasmatic protein, was determined by means of using the Protein Efficiency Ratio (PER) and the Apparent Digestibility (AD), following the methods proposed by the AOAC (2004). To do so, 20 recently weaned Sprague Dawley male rats were used. They were divided into groups of 10 and placed in individual galvanized wire-net-floor cages, under the same environmental conditions, which are temperature, air and lighting (12 h light/12 h darkness). Before starting the research, the rats were acclimatized for 3 days; one group was fed a controlled diet based on casein and the other was fed with the product made with tender corn and plasmatic protein, previously dehydrated at a temperature of 100ºC on a 3536 LAB-LINE™ conventional stove.

The food and water intake was provided ad libitum. The apparent digestibility was measured during the first ten days of the research; while the protein efficiency rate was taken on the 28th day.

Sensory assessment: A untrained panel of 215 children of both sexes, whose ages ranged from 10 to 14 chosen from the fifth and sixth grade of a local elementary school; assessed cooked corn pancakes for two sensory attributes colour and taste for degree of likeness. White plastic plates labeled with four digit codes from a random number table were used to conceal the identity of the corn pancakes samples. Samples (50 g) were served in random order to the child, isolated in partitioned booths illuminated with white fluorescent light.

The product was offered once a day, during school week, to each fifth and sixth grade section; in both, morning (9:00 AM) and afternoon (3:00 PM) sessions. Water was provided to each child as a means for cleansing and rinsing their palates between each sample. All data was recorded on an instrument designed for such purpose using a 5-point hedonic scale. The evaluation scale was as follows: I like it very much, I like it, it makes no difference to me, I do not like it very much and I do not like it at all.

Microbiological analysis: Eleven grams from each sample were aseptically weighed into sterile jars. Samples were blended for 2 min at high speed after the addition of 99 mL of 0.1 peptone (Oxoid, Basingstoke, UK) solution. Aliquots (1 mL) of sample were serially diluted in 9 mL of sterile 0.1 peptone solution. Sevenfold serial dilutions were carried out. Each dilution was spread-plated and duplicated, on plate count agar (Difco Laboratories, Sparks, Md) for enumeration of total viable counts at 35ºC for 24 to 48 h. Petrifilm plates were used to determine (in duplicate) coliform count, *E. coli* count and fungus and yeast. The procedures followed were those developed and outlined by 3M™ (St. Paul, Minn). The manufacturer's written instructions were followed. Bacterial counts were transformed to logs.

Table 2: Mean values (g/100) of cooking yield and chemical composition of corn pancakes

Attributes	Formulation A*	Formulation D*
Cooking yield	77.17±0.16 [b]	80.74±0.16 [a]
Humidity	58.58±0.12[b]	63.28±0.12[a]
Protein	3.54±0.04 [b]	6.47±0.04 [a]
Fat	0.93±0.02	0.98±0.02
Fiber	1.45±0.04	1.40±0.04
Carbohydrates	26.03±0.02	26.97±0.02
Ash	0.80±0.02	0.90±0.02
Caloric energy (Kcal.)	126.65±0.04 [b]	136.82±0.04 [a]

Means with different superscript differ significantly ($p<0.05$) as indicated by LSD procedure; * (A: Control; D: Product formulated with plasmatic protein)

Statistical analysis: The statistical analysis of the final results was performed using a mixed model, which included the fixed effect and the random effect of the sample on the observations. The differences between means were obtained with T student tests. Data was analyzed by the general linear model procedure of the Statistical Analytical System (SAS PROC GLM, 1997). The predetermined acceptable level of probability was 5% ($p<0.05$) for all comparisons.

RESULTS AND DISCUSSION

The D formula was selected out of all formulas; since it allowed the greatest addition of plasma (40%), without affecting the technical handling of the mixture or the organoleptic characteristics of the final product. Table 2 shows the mean values regarding cook yield, moisture, protein, fat, raw fiber, carbohydrates, ashes and caloric energy for formulas A (without plasma) and D (with plasma).

Cook yield: Significant differences were found between the two of them ($p<0.05$). The formula with plasma presented the highest yield (80.74±0.16 g/100) while the one without plasma had lower yield (77.17±0.16 g/100). The higher yield, given by the product with plasma, could be attributed to the binding properties of the plasmatic protein. Barboza *et al*. (1996), point out that the positive effect, the bovine plasma has over the yield and the binding action, is due to its unique gelation property. In addition, if it is used at a protein concentration of 4.5%, this property will not be affected by refrigerating or freezing temperature.

Many researches have confirmed the gelation property of proteins to be a functional one, essential in the preparation of various types of food; since it increases water absorption, thickness, stickiness and emulsion. Márquez *et al*. (1997) point out that the use of plasma instead of water, when preparing meat products, improves their yield; since the gel it produces helps retain fat and water, thus reducing product loss at the moment of cooking.

Table 3: Essential amino acid profile (g/100g of protein) of product formulated with plasmatic protein and tender corn and amino acids profile reported by FAO/WHO

Amino acid	Product with plasmatic protein	FAO/WHO (1991)* (standard)
Histidine	3.89±0.27	1.9
Isoleucine	3.82±0.33	2.8
Leucina	9.02±0.42	6.6
Lysine	5.72±0.20	5.8
Methionine	1.60±0.45	2.5**
Phenylalanine + tyrosine	7.22±0.64	6.3
Threonine	4.43±0.33	3.4
Valine	4.02±0.42	3.5

Food and Agriculture Organization/World Health Organization; **: Methionine + cisteíne

The gel-forming ability upon heating is probably the most interesting attribute of plasma, because many food products go through a thermal treatment prior to commercialization. Under appropriate conditions, a three-dimensional network may be formed, contributing to the development of the internal structure of the foods such as desirable texture characteristics and improved properties like water holding capacity (Hermansson, 1982). It has been shown that protein interactions played a key role in the development of the gels physical properties and this behavior had to be taken into account particularly because the use of plasma proteins as ingredients in the food industry is influenced by the proportion of each major constituent in the formulated functional products (Davila *et al*., 2007).

Moisture, proteins, fat, raw fiber, carbohydrates and caloric energy: A significant difference ($p<0.05$), regarding moisture, was found between the two formulas. The product with plasma showed a higher moisture content (63.28 g/100); it was probably due to the fact that the proteins found in the plasma denature during cooking, thus causing the product retain additional humidity and therefore making it softer. Therefore, retention of water in corn pancakes is important since excessive water loss makes an unacceptable product.

Fulfilling all expectations, the results of this research indicated there were significant differences between the two formulas, regarding protein content ($p<0.05$). The product with plasma showed a have higher protein content (6.47±0.04 g/100), while the one without plasma showed to be (3.54±0.04 g/100). This could be attributed to the fact that the addition of plasma increases the low protein content of the product. This indicates that the addition of plasma, which is high in the amino acid lysine, produces a positive nutritional effect in the resulting product. Including this product in the children's diet would contribute to satisfy the standard protein daily requirements.

From the data shown in Table 2, there was no significant difference between the two products in fat content; this could be attributed to the minimum concentration of fat found in plasma and the low percentage (1%) of it found in tender corn (Bressani and Mertz, 1988). This research revealed that the product with addition of plasma represents an important source of fiber (1.40 g). The content of ashes was similar in both formulas.

Table 4: Protein quality of corn pancakes prepared with tender corn and plasmatic protein

Parameters	*Control diet	Experimental diet
Apparent digestibility (%)	78.94	81.08
PER	3.03^{a}±0.80	2.64^{b}±0.78

Means with different superscript differ significantly ($p<0.05$) as indicated by LSD procedure Diet based on casein

Amino acids: Once the results, obtained from analyzing the product with plasma, were compared to the profile of essential amino acids (Table 3), proposed by the Food and Agriculture Organization/World Health Organization, it was clearly shown that they are above the standard requirements; with the exception of methionine, which could not be compared to the parameter established by the (FAO, 1991), since these institutions report its value as the sum of sulfur amino acids (methionine + cysteine). As it was indicated previously, the corn quality is limited due to its deficient in of certain essential amino acid, especially lysine and tryptophan; however, it contains sulfur amino acids such as methionine and cysteine (Bressani and Mertz, 1988).

Animal blood proteins are similar to egg proteins, which are considered well balanced, where the essential amino acids are found in adequate proportions; however, due to the deficiency in isoleucine and methionine found in the blood, it should be supplied along with other proteins which can compensate for the deficiency of these amino acids (Marquez *et al*., 2005). This research determined that the combination of methionine and lysine found in corn and plasma respectively, helped obtain a product with a higher protein value.

Biological evaluation: Table 4 shows the apparent digestibility and the protein efficiency ratio of the product with plasma. According to the results, experimental diet proteins are very digestible; considering that out of the total protein ingested (4.30 g) only 18.92% was excreted while the rest (81.08%) was digested. Oropeza and Ortiz (1989) reported lower digestibility values (66.54 and 70.25%), compared to the ones reported by this research, after testing several tender hybrid corn kernels; while other authors have reported a plasma digestibility that ranges between 83 and 92% (Del Rio de Reys *et al*., 1980).

The Protein Efficiency Ratio (PER) obtained in this research, shows that the animals fed with the experimental diet, experienced a weight gain of 2.64 g per each g of protein ingested; which confirms that the proteins used in the formula are capable of sustaining and helping the growth of young animals.

Oropeza and Ortiz (1989), after evaluating the kernel's protein quality of several corn hybrids through

Table 5: Sensorial evaluation of corn pancakes prepared with tender corn and plasmatic protein

Degree of likeness percentage/formulation				
Scale taste colour	D *%	A * %	D *%	A* %
I like it very much	88.4	87.9	95.3	94.9
I like it	10.2	8.8	4.7	4.2
It makes no difference	0.9	3.3	0.9	0.9

A: Control; D: Product, with tender corn and plasmatic protein

Table 6: Mean values (log cfu/g) for aerobic plate count (RTA), E. *coli* and fungus and yeast (HL) in corn pancakes prepared with tender corn and plasmatic protein

Days	RTA	E.C	H L
0	2.52^{a}±0.04	<1±0.0	$<1^{a}$±0.01
1	4.02^{b}±0.02	<1±0.0	1.24^{b}±0.02
2	4.45^{b}±0.04	<1±0.0	2.56^{c}±0.04
3	5.00^{c}±0.02	<1±0.0	3.26^{d}±0.03
4	5.99^{c}±0.02	<1±0.0	3.78^{d}±0.03

a, b, c, d: Means with different superscript differ significantly ($p<0.05$) as indicated by LSD procedure

biological and chemical tests, using casein as a control agent, reported values of 1.57 and 1.74 for corn and 3.41 for casein control. When comparing these results to the ones obtained in this research (PER 2.64), it is evident that the addition of plasma improves considerably the protein efficiency.

Sensorial evaluation: The result of the degree of likeness test show no significant difference between the two formulas; furthermore it was shown that out of all the children who formed the tasting panel, 88.3% accepted the product for its taste, while 95.3% did it for its color (Table 5). The addition of plasmatic protein to the formula, did not affect the taste or the color. It is important to emphasize that none of the children showed total displeasure for the product.

Even though the texture was not evaluated, it was shown that the plasma gave the product better consistence and softer texture; thanks to the functional properties of the plasma, which as well as the albumin and globulin, found in egg white make the air intake easier during the whisking process. Bovine blood plasma proteins are equivalent in functional aspects to egg albumen (Lee *et al*., 1991) providing heat coagulation properties similar to natural egg in cereal products (Duxbury, 1988).

Microbiological evaluation: Table 6 shows the results of the microbiological growth on the corn pancakes during storage under condition evaluated. The initial total viable count was 2.52±0.04 log ufc/g at day 0, which indicates an acceptable initial microbiological quality and no fungus, yeast, or *E. coli* were found. These values increased during storage; reaching on the fourth day, values of 5.99±0.02 log ufc/g for aerobics and 3.78±0.03 log ufc/g for fungus and yeast. This helped establish the shelf life of the product, which can be estimated to be 3 days. The results of this research were interpreted based on acceptability limitations selected from previous researches as well as some recommended norms (Curtis *et al*., 2000).

CONCLUSION

After evaluating the results obtained related to the product acceptability and protein quality, not only for having the right amount of essential amino acid but for its excellent digestibility and PER, it is evident that this product could be used as a means of resolving the problem of malnutrition associated with the consumption of cereal-based foods due to their low protein content. The plasma gave the product better consistence and softer texture; thanks to the functional properties of the plasma. Corn fortification with plasma has led to improvements of the protein quantity and quality of this food.

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