A PROTEIN CONCENTRATE FROM DISTILLERY YEAST, AND ITS APPLICATION TO SUPPLEMENT CORN TORTILLAS

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SUMMARY

The purpose of this study was to increase the protein quality of tortillas supplementing them with a protein concentrate obtained from the yeast *(Saccharomyces cerevisiae)* cream from an alcohol distillery.

Rupture of the yeast cell wall was studied, and a procedure for obtaining a high percentage of broken cells from the yeast was developed. A protein concentrate was obtained from the cell-wall free portion. Techniques were developed to obtain the protein concentrate with a low level of nucleic acids.

The latter were reduced 91%, and the protein increased 55% with respect to the initial yeast.

Different levels of protein concentrate were added to dough made from corn of the Nutricta variety. The resulting tortillas were submitted to a taste panel for sensory evaluation, and the results were statistically analyzed. The highest level of supplementation that did not affect negatively acceptation by the panel was 18% dry weight.

In the tortillas with the highest acceptable level of supplementation, protein content increased 60% when compared to the control tortillas, and a significant improvement in lysine content was observed. Nucleic acid content was low, having been reduced to levels which did not represent a limiting factor for the use of yeast in a product destined for human consumption.

INTRODUCTION

In some areas of Latin America there exists a protein shortage and deficiency in the human diet, mainly due to nutritional habits, as well as to

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availability and price of food. In northern Central America and Mexico, corn is the main source of food energy and vegetable protein in the low-income population groups, being consumed essentially in the form of tortillas.

Although corn protein is deficient in lysine and tryptophan (1), new improved varieties have been developed through plant breeding techniques.

Another approach has been to complement and enrich the nutritional value of the protein through mixing with other food materials and developing processed food products (2). Many vegetable protein sources have been used as supplements in the past; however, few efforts have rather been directed towards the possibility of employing microbial biomass from different microorganisms. A significant improvement in the protein quality of lime-treated corn was obtained by the addition of 3% Torula yeast (3).

The industrial processing technology for producing microbial biomass from a series of different raw materials has favored the development of the continuous system of submerged cultivation in stirred tank bioreactors and the separation of the microbial biomass solid. Among microorganisms, yeast has been the most studied and several industrial factories in the world produce such products (4, 5). The major limitation in their future expansion is economic since its selling price must be competitive with alternative sources.

The protein content of yeast varies according to the strain, the substrate and growth conditions, and is approximately 50%; nutritionally it is low in sulfur amino acids as is typical for all microorganisms (6). Its major drawbacks are a cell wall which interferes with protein digestion (7, 8), the presence of antigenic and allergic compounds (9) and a rather high (6-16%) nucleic acid content (6).

Hence, in order for yeast to be incorporated into human food products, certain processing steps are required to circumvent the above-mentioned problems. Cell wall rupture and protein concentration can be done by mechanical, chemical and enzymatic procedures alone or in combination (10, 11), and nucleic acid separation can also be incorporated into such processing schemes.

The objective of the present work was to obtain a yeast protein concentrate, low in nucleic acid, suitable to be mixed with corn dough for tortilla preparation. The maximum amount that could be added without impairing the organoleptic characteristics of the finished product was established also.

MATERIAL AND METHODS

Yeast Biomass

The yeast employed in the experiments was obtained directly from a local rum factory fermenting a mixture of virgin and blackstrap cane molasses. It was an ethanol efficient *Saccharomyces cerevisiae* strain. At the factory it had been acid treated, washed and centrifuged; the paste was brought to the laboratory and was then freeze-dried.

Cell-Wall Breakage

All the tests for breaking the yeast cell wall were done by mechanical friction, employing a laboratory glass bead disintegrator operated at 4°C. The bead diameter was 0.3–0.5 mm.

Protein Concentration

Basically, the methodology of Hedenskog and Mogren (11) was followed. The cell suspension from the mechanical cell wall disintegrator was centrifuged until a clear supernatant was obtained. The pH was adjusted to 9.5, a volume of a 4.2% NaCl solution was added, and the mixture was heated rapidly to 80°C and kept at this temperature for only 8 s. The precipitate was centrifuged at 10,000 rpm and 4°C, washed several times with water, and freeze-dried.

Tortilla Manufacture

The detailed description of this operation has been published elsewhere (12), and the improved Nutricta variety was used.

Organoleptic Evaluation

Tortillas made with corn and corn-yeast and corn-protein concentrate were evaluated for flavor, color, aroma and texture, with a previously-trained panel following standard procedures (13). The results were statistically evaluated at 1 and 5% of significance (14).

Chemical Analysis

Moisture and nitrogen were determined by the AOAC methods (15, 16); protein hydrolysis for amino acid determination according to Kaiser *et al.* (17); amino acid analysis by liquid chromatography in an autoanalyzer; and nucleic acids, according to Sarwar and Shah (18).

Protein Efficiency

This was determined from the amino acid analysis, employing the equations of Alsmeyer, Cunningham and Happich (19).

RESULTS AND DISCUSSION

Results obtained for cell-wall breakage are presented in Table 1, where it is seen that with 10% yeast suspension at pH 9.5, a cell wall breakage of 97% was obtained after a disruption time of 25 min. A succesful mechanical treatment, as the one obtained here, has its advantages over alternative procedures, either chemical or enzymatic, as it can be scaled up, and its operation optimized (10, 21). Cell-wall separation has many advantages: it increases nitrogen extraction (8), eliminates coloring reactions and compounds that might originate gastrointestinal problems (5, 22) and improves digestibility (23).

TABLE 1

CELL WALL BREAKAGE

Effect of biomass concentration

(Disruption time, 15 min; pH = 7)

Yeast concentration o/o	Broken cells o/o
5	21.55
10	79.60
16	69.85
25	65.05
50	33.00

Effect of pH

(Disruption time, 15 min; yeast concentration, 10o/o)

pH	Broken cells o/o
6.5	79.60
9.5	87.52
11.0	83.17

Effect of disruption time

(Yeast concentration, 10o/o; pH = 9.5)

Time, min	Broken cells o/o
20	93.78
25	97.00
30	95.61
35	94.52

It also presents processing disadvantages, as it reduces the protein yield and adds another unit operation to the process (24, 25). In our case it was a necessary procedure in order to improve acceptability of the product.

Figure 1 illustrates the experimental procedure followed to obtain the protein concentrate once the cell wall was separated. Treatment of yeast cells with alkali pH 11 weakens the cell wall, and facilitates the rupture by mechanical methods (26). Hedenskog and Mogren (11) recommended a pH of 11.5, but we found that at 9.5 the protein precipitated in a more

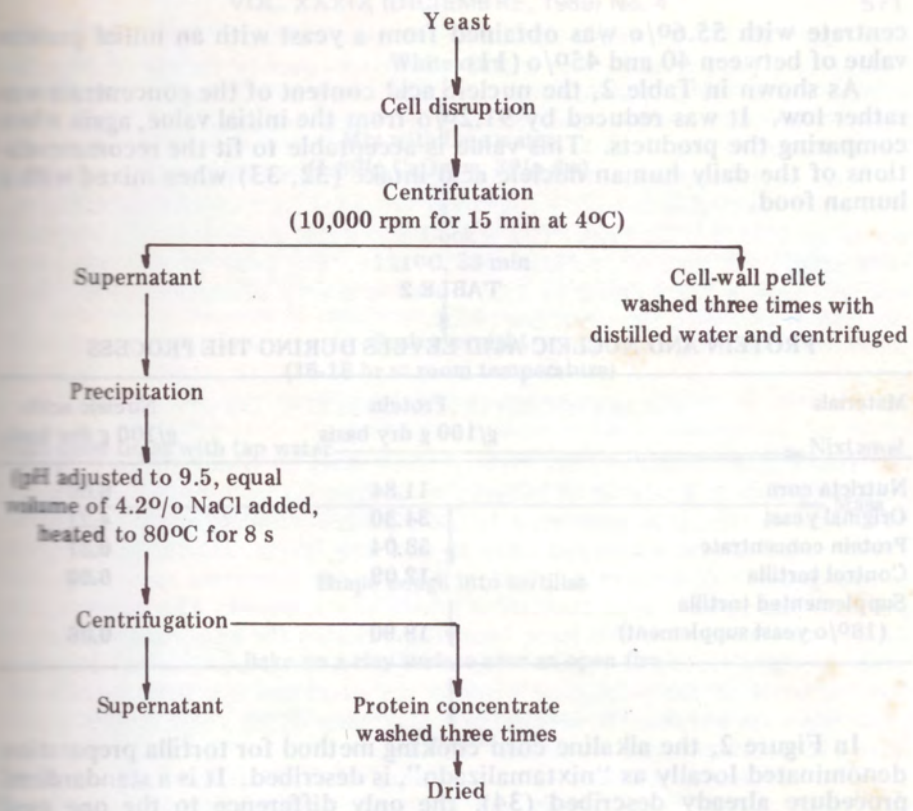

FIGURE 1

Preparation of protein concentrate

firm way which favored its ultimate recovery. Less drastic alkaline conditions improve the membrane permeability, increasing protein solubility and reducing nucleic acids (27, 28). A low NaCl concentration was used, as no further improvements were obtained at higher values. The presence of salt improved coagulation of the protein precipitate, as reported elsewhere (24, 27). Heat was needed to activate the endogenous yeast ribonuclease (29, 30). The activation may occur by changes in cellular location of ribonuclease, by conformational changes in ribonuclease itself, or via the denaturation of ribonuclease's inhibitor (31). Cell-wall washings improved protein recoveries by about 10%.

A protein yield of 65.3% was obtained after cell-wall breakage. The precipitated protein represented 97.8% of the amount in solution.

The protein content of the concentrate was 53.0% on a dry basis. The fresh yeast had 34.3%; hence, a protein increase of 54.6% was obtained when comparing the two products. In the literature, a con-

centrate with 55.6o/o was obtained from a yeast with an initial protein value of between 40 and 45o/o (11).

As shown in Table 2, the nucleic acid content of the concentrate was rather low. It was reduced by 91.2o/o from the initial value, again when comparing the products. This value is acceptable to fit the recommendations of the daily human nucleic acid intake (32, 33) when mixed with a human food.

TABLE 2

PROTEIN AND NUCLEIC ACID LEVELS DURING THE PROCESS

Materials	Protein g/100 g dry basis	Nucleic acids g/100 g dry basis
Nutricta corn	11.84	0.00
Original yeast	34.30	4.21
Protein concentrate	53.04	0.37
Control tortilla	12.09	0.00
Supplemented tortilla (18o/o yeast supplement)	18.90	0.06

In Figure 2, the alkaline corn cooking method for tortilla preparation denominated locally as "nixtamalizado", is described. It is a standardized procedure already described (34), the only difference to the one used commercially being the amount of calcium employed. The corn dough moisture in the process was 63.2o/o and for the tortilla 49.8o/o; hence, some water is lost during tortilla cooking.

Fresh yeast corn mixtures were used to prepare tortillas employing the procedure described. The amount of yeast was varied between 2.4, 4.8, 7.2, 12 and 24o/o of the corn on dry-weight basis, (1, 2, 3, 5 and 10o/o on a wet-weight basis, respectively). The resulting products were compared to an only corn control in organoleptic tests. The data revealed that 4.8o/o was the maximum amount of yeast that could be added to the product without impairing its organoleptic characteristics. Similar results had been obtained previously by Bressani (35).

Another set of data was obtained employing the protein concentrate at the levels of 6, 12, 18 and 19o/o on a dry-weight basis (2.5, 5, 7.5 and 10o/o wet-weight basis, respectively). The same type of organoleptic evaluations showed in this case that it was possible to incorporate up to 18o/o without establishing differences between the enriched products and the control.

In Table 2 it is easily seen that this consumer-acceptable tortilla had 18.9o/o of protein, figure which is 59.7o/o higher than the control product. The amount of nucleic acids in this product is quite low.

Table 3 illustrates lysine content for the different materials and products; the higher lysine content of the enriched tortilla over the control, is statistically significant at the 0.05 level. If normal corn had

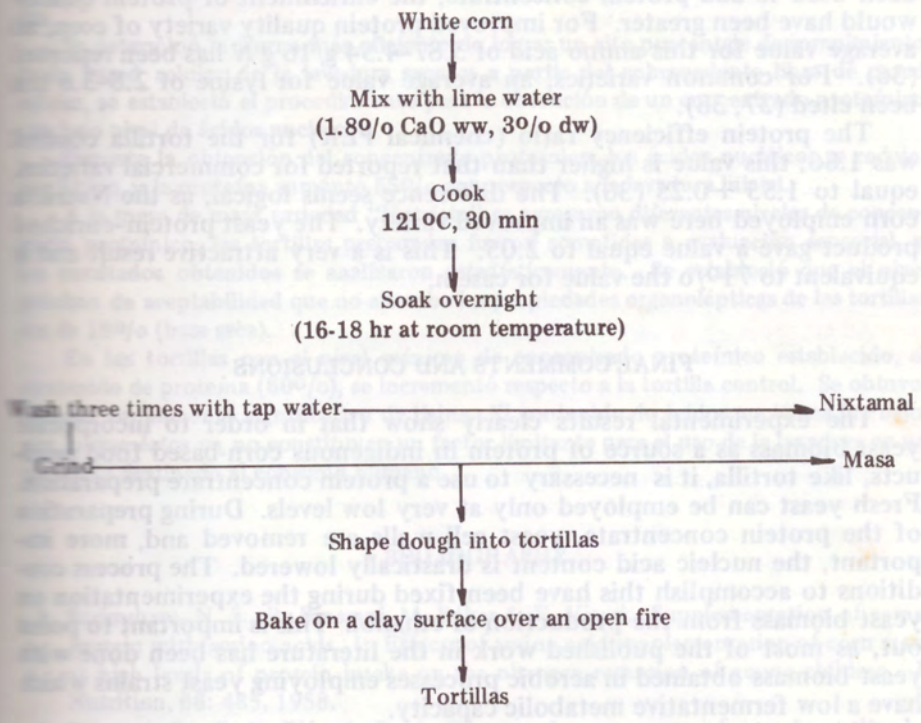

FIGURE 2

Nixtamalization process used

TABLE 3

ESSENTIAL AMINO ACIDS
g/100 g protein

	Protein concentrate	Tortilla control	Tortilla 10% protein concentrate	Yeast	Nutricta corn
Isoleucine	5.61	1.48	2.05	2.55	0.81
Leucine	8.94	5.29	5.59	4.39	5.71
Lysine	8.99	2.63	4.15	6.86	2.53
Methionine	0.69	0.36	0.50	0.22	1.75
Phenylalanine	4.09	1.58	1.65	1.62	1.48
Threonine	3.87	1.57	1.96	2.70	1.28
Valine	6.81	3.03	2.52	4.05	3.42

been used to add protein concentrate, the enrichment of protein quality would have been greater. For improved protein quality variety of corn, an average value for this amino acid of 3.87–4.54 g/16 g *N* has been reported (36). For common varieties, an average value for lysine of 2.8–3.6 has been cited (37, 38).

The protein efficiency ratio (chemical PER) for the tortilla control was 1.86; this value is higher than that reported for commercial varieties, equal to 1.55 + 0.25 (36). The difference seems logical, as the Nutricta corn employed here was an improved variety. The yeast protein-enriched product gave a value equal to 2.05. This is a very attractive result and is equivalent to 71o/o the value for casein.

FINAL COMMENTS AND CONCLUSIONS

The experimental results clearly show that in order to incorporate yeast biomass as a source of protein in indigenous corn-based food products, like tortilla, it is necessary to use a protein concentrate preparation. Fresh yeast can be employed only at very low levels. During preparation of the protein concentrate, yeast cell walls are removed and, more important, the nucleic acid content is drastically lowered. The process conditions to accomplish this have been fixed during the experimentation on yeast biomass from the production of ethanol. This is important to point out, as most of the published work in the literature has been done with yeast biomass obtained in aerobic processes employing yeast strains which have a low fermentative metabolic capacity.

The maximum amount of concentrate was 18o/o of the corn on a dry-weight basis. The tortilla had the same organoleptic characteristics as the control, and showed improved nutritional properties.

The technology that was developed could easily be adapted in Guatemala and other tortilla-consuming countries as distiller's yeast is available and, more important, the protein concentrate could be added during the industrial production of instant tortilla flour.

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RESUMEN

OBTENCION DE UN CONCENTRADO PROTEINICO A PARTIR DE LEVADURA PROVENIENTE DE UNA DESTILERIA, Y SU APLICACION COMO SUPLEMENTO DE TORTILLA DE MAIZ

El objetivo de este estudio fue el de aumentar la calidad proteínica de las tortillas, suplementándolas con un concentrado proteínico obtenido a partir de crema de leva-

tura (*Saccharomyces cerevisiae*) proveniente de una planta procesadora de alcohol y ron.

Se determinó la forma más eficiente de lograr un alto porcentaje de rompimiento de la pared celular de la levadura seca, y a partir del sobrenadante libre de pared celular, se estableció el procedimiento para la obtención de un concentrado proteínico con bajo nivel de ácidos nucléicos.

Durante la obtención del concentrado proteínico, los ácidos nucléicos se redujeron 91%, y la proteína aumentó 55% con respecto a la levadura inicial.

A la masa de maíz variedad "Nutricta" se agregaron diferentes niveles de concentrado proteínico, las tortillas preparadas fueron sometidas a evaluación sensorial, y los resultados obtenidos se analizaron estadísticamente. Se estableció que el nivel máximo de aceptabilidad que no alteraba las propiedades organolépticas de las tortillas era de 18% (base seca).

En las tortillas con el nivel máximo de concentrado proteínico establecido, el contenido de proteína (60%), se incrementó respecto a la tortilla control. Se obtuvo, además, un aumento significativo de lisina. El contenido de ácidos nucléicos fue bajo, por lo que éstos ya no constituyen un factor limitante para el uso de la levadura en un producto destinado al consumo humano.

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