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INTRODUCTION

The report will be divided into four areas: Section 1, work that has been done on the extrusion cooking of mixtures of wheat, sunflower, fish protein concentrate, and mixtures of these; Section 2, nutritional, acceptability, and toxicological studies on extrusion processed products; Section 3, the work in progress in the scaling up of detoxification processes for rapeseed meal and pilot scale processing of rapeseed protein isolate; and Section 4, development of other protein sources.

1. Extrusion Processing of Cereal Mixtures

a. Alteration of Process Conditions

Processing variables for mixtures of skim milk, whole wheat flour, low fiber sunflower meal, and fish protein concentrate were studied during the period. Variations in processing conditions as well as the addition of fat and flavor as part of the mixtures were also analyzed.

The effect of different temperatures of extrusion upon the characteristics of the product of mixtures were determined. It was evident that the method of measuring temperatures in the expander supplied by the manufacturer were unsatisfactory. In order to more accurately determine the actual product temperature in the output section of the expander, a series of thermocouples were imbedded in the die-plates. Previously, measurements had been made using a thermocouple attached

to the jacket as supplied by the manufacturer, or measurements were taken by attaching thermocouples to the barrel. The difference in temperature between these measuring points exceeded 15°C . which, at the low temperatures found necessary for the extrusion of wheat-milk mixtures, represented a sizable error in the measurement of product temperature and, thus, with an error of this size made control difficult, since the error was variable depending upon the amount of cooling supplied to the jackets.

In all runs the die area was maintained constant at 0.58 cm^2 . The product was removed from the cutting section of the expander and dried on stainless steel wire trays in a drying unit which was constructed in the last six-month period.

b. Effect of Size on Rehydrateability of Product

The expanded product is to be utilized in the feeding of infants and children and is designed to be made up with water. The ease of rehydrateability thus is an important problem. Using a single extrusion temperature as a standard, e.g., 250°F ., the relationship between rehydrateability and size of particles was investigated. Results suggest that for the product Leche Alim without added fat a grind size of a 60 mesh sieve gave excellent rehydrateability. Flour grinds such as may be obtained by Alpine milling cause lumping during preparation, and larger grinds were difficult to rehydrate. Investigation of other mixtures also indicated that a mesh size around 60 gave the best

rehydrateability to a fully gelatinized product. As a consequence, preparations for testing are uniformly ground to 60 mesh.

c. Effect of Process on Physical Properties

The effect of processing on the physical characteristics utilizing a 60 mesh product rehydrated in water with a composition as shown in Table 1 was studied. Extrusion was at 250^oF., and the product qualities were compared to those of expanded wheat. The consistency, water absorption index, and water solubility index is shown in Table 2. Under optimum processing conditions it is obvious that the consistency of the fully-processed Leche Alim produces a much thicker suspension in water than does the unprocessed material. This is obviously due to the high degree of glutinization of the starch. From a physical standpoint this is of importance since the thicker material is capable of holding the different components of the formula in suspension, and a gruel prepared from the product is stable for 2 to 4 hours.

Table 3 shows the nitrogen solubility of processed Leche Alim vs. unprocessed. The sodium hydroxide solubility was measured by the method of Lyman et al. (J. Nutr. 49, 679, 1953). While the processed material decreased its nitrogen solubility in water, solubility in sodium hydroxide increased, thus illustrating that not only is the starch glutinized in the process but, additionally, there is some protein modification. As the section on nutritional evaluation indicates, this does not in the least change the protein availability in diets.

Using the conditions which give the best nutritional value for the product, the viscosity of the processed and unprocessed Leche Alim was measured in a viscograph-amflograph at 30°C. The unprocessed or non-gelatinized Leche Alim gave effectively a 0 viscosity in Brabender units, while processed Leche Alim gave a viscosity of 180 units; thus illustrating the increase in consistency of viscosity of the fully-gelatinized product.

d. Stability and Fat Addition

The stability of the pre-cooked Leche Alim is excellent as reported in previous progress reports. In order to increase the caloric density of the product three lots were formulated with 4% hydrogenated fish oil. These were stored for six months in open containers. After 26 to 28 weeks peroxide values were measured on the product. The results are shown in Table 4.

It is apparent that even utilizing hydrogenated fish oil, the product shows excellent stability. The reason for the difference between the various lots in peroxide values is not apparent but, in any case, the values obtained are extremely low. Thus, this type of product (that is, an extruded pre-cooked product) containing significant percentages of fat is quite stable. In closed containers, the hoped-for shelf life of one year should be easily obtainable.

In addition to using hydrogenated fish oil in the product, sunflower oil which is readily available on the Chilean market was in-

corporated into Leche Alim at a 3.3% concentration. This is presently undergoing nutritional evaluation in infants, and the results of this will be reported in the next progress report.

e. Sensory Evaluation

Unflavored Leche Alim made by using low-temperature expansion (to maximize both nutritional and physical characteristics) was compared with unprocessed Leche Alim by sensory panels. The two products were presented to a taste panel of 100 school children between the ages of 7 to 15 years. The two products were presented as a suspension at 15% weight/volume in cold water. The formulation which had been used in the distribution of the unprocessed product to populations was used. The processed Leche Alim, because of its gelatinization, was much thicker than the unprocessed material and did not show any tendency to separate. This factor made it preferred by 97% of those tasting.

To investigate the effect of flavoring on Leche Alim a series of flavored formulations were compared against unflavored or cereal products. The panelists utilized for flavor ranking were school children aged 12 to 14 years. Approximately 25 children were utilized in the ranking for appearance and approximately the same number were used for flavor. The tests were replicated utilizing the same subjects on different days in order to substantiate the results.

The method used for the analysis was that of paired comparison by association of two factors or attributes; that is, flavor and appear-

ance. Finally, multiple comparisons were made of all samples. The rating system on flavor was the seven point Hedonic scale and ranking was used for appearance.

Coloring matters used in the trial were added by spraying diluted color solutions into the pre-mixed assembly on the expander, using air pressure for atomizing liquid. This resulted in good distribution prior to expansion. The colorings used were those approved by Chilean regulations.

Flavoring components were added to the dry mix as encapsulated beads before pre-conditioning. A large number of flavoring compounds were tested to determine which were adaptable to this method of addition. Four of the flavoring components would show little or no loss during processing, and these were the ones submitted to the flavor panels.

The actual data on this was submitted as a supplemental report in February 1970, although the third replication was not finished. The data indicates without a doubt that flavoring and coloring the expanded product makes it much more acceptable to children than the expanded unmodified product. The expanded product, however, is considerably more desirable than the raw mix.

An acceptability trial of the unground processed Leche Alim flavored but uncolored was done utilizing approximately 125 school children between the ages of 8 to 14 years. Simultaneously, a comparison

was made between milled Leche Alim and the unground material eaten out of hand. The following conclusions were drawn from this study:

- a. The product that could be eaten out of hand (not mixed with water or milk) was preferred in this age group.
- b. In a comparison in which sugar was added to the product, the best acceptability was achieved with the addition of 7 to 8% sugar.
- c. The cereal flavor (reinforced wheat flavor) was preferred above those of strawberry, butter taffy, unflavored, or almond.

2. Nutritional, Acceptability, and Toxicological Studies

a. Nutritional Evaluation of Process Variables

By reducing the temperature of expansion and modifying the feed and water inputs, it was possible to develop considerably higher nutritional values of the expanded product. The experimental work done with the expander in the past period permitted us to determine the best conditions for operation. The variation in rate of feed, speed of the mixers, the amount of water added to the mixture, as well as the speed of pre-conditioning were studied. At the present time, expansion is performed with minimum pre-conditioning and with cooling on the barrels of the extruder. By inverting the flow of water compared to that of steam heating, it is possible to operate at comparatively low barrel temperatures. With such an arrangement, the pilot scale unit is capable of producing 300 pounds per hour of cereal-based product with a total retention time of 9 seconds. Thus, even the pilot scale unit operating eight hours a day under these conditions

is capable of producing over 300 tons of product per year. In the previous report we indicated that the protein efficiency ratio of processed material was approaching that of the raw material and, consequently, little or no nutritional effectiveness was apparently lost.

The results operating under the presently determined conditions indicate a vastly superior product from a nutritional standpoint. Table 5 shows that the processed Leche Alim is significantly better than the unprocessed product which itself exceeds casein. The mixture of wheat, sunflower, and fish protein concentrate processed by the expander to which milk solids have been added is not significantly different in P.E.R. than that of the entire mixture passed through the expander.

The results of a large number of feeding trials performed within the last year is thus fully justified by the fact that, by using nutritional efficiency to determine the effect of variation in process, it was possible to adjust the process to give a nutritional efficiency equivalent to or higher than the raw mix. As far as our group is concerned, this is the first time that nutritional efficiency (P.E.R.) has been used as a method of determining industrial processing conditions.

It is thought that the increase in protein efficiency ratio is due to an increase in digestibility or absorption of the gelatinized starch mixture; this born out by other feeding trials where it was possible

to gelatinize the starch mixture, add the protein sources to it, and achieve higher protein efficiency ratios than that of the uncooked or non-gelatinized mixture.

In order to better determine the mechanism of this increase, NPU's and digestibility studies are proposed and will be undertaken in the next period.

b. Acceptability of Milk Substitute in Curico

As indicated previously, the experiment of wide-scale distribution of Leche Alim was initiated in December 1968 in the outskirts of the city of Curico. The number of children that were to be supplemented was 1,100 from 0 to 5 years of age. An equivalent control group was included. In cooperation with the "Centro de Madres" four centers were utilized in the distribution scheme. Children of pre-school age were allotted 1.5 kilos of Leche Alim. The mother of the family came to the distribution center once a month and, on that occasion, the height and weight measurements of the children were determined. The personnel required for this operation were recruited from the personnel normally engaged in the local distribution centers, and their salaries were paid by the National Health Service.

After three to four months of distribution, a survey was made whose objective was to check the acceptance of the new product by the mothers. In addition, an attempt was made to find the use to which the mothers put the product and to determine the acceptability or

rejection on the part of the child. In the experiment any propaganda or promotion of the product was avoided in order not to influence the mothers. The results of this survey have been calculated at the present time and are presented below.

The distribution was carried on for a period of 18 months ending in the spring of 1970. At mid-point and at the end of the distribution, surveys were made to again determine the long-term acceptability of the product. These results are still in the process of being assembled, and will be forwarded to the Agency as soon as they become available.

The preliminary data from the 18-month study indicates that the formulation was moderately well-accepted over the 18 months and that the height and weight gains of the population were equivalent to those that were receiving milk. In any case, this data will be submitted for publication and forwarded to the Agency as soon as the completed correlation of the interviews with the people are available.

The data over the three-month feeding period is summarized below in a series of 11 questions.

1. Question - Do your children like Leche Alim? (each person indicating the age of the child or individual).

Answer - (In percentage)

<u>Age</u>	<u>Yes</u>	<u>No</u>	<u>Don't know</u>
1-4	59%	39%	1%
4-7	62%	37%	1%
7-10	63%	34%	3%
10-13	55%	41%	4%
<u>13-15</u>	<u>56%</u>	<u>41%</u>	<u>3%</u>

2. Question - Do you have any trouble preparing it?

Answer - (In percentage)

Yes	No
14%	86%

3. Question - If you have trouble preparing it, what kind of trouble is it?

Answer - (In percentage)

It is hard to dissolve, it forms into balls	2%
They prefer dry powder	3%
They don't know	8%

4. Question - Do you like "Leche Alim"? (generally the mother was the informant)

Answer - (In percentage)

Yes	No	I have not tried it
65%	33%	12%

5. Question - Does your husband like it?

Answer - (In percentage)

Yes	No	He has not tried it
60%	38%	12%

6. Question - Do other adults at home like it? (specifying each one)

Answer - (In percentage)

Yes	No	They have not tried it
28%	53%	19%

7. Question - Do you like "Leche Alim"? Cold? Hot? With sugar?

With no sugar?

Answer - (In percentage)

Cold	12%
Hot	68%
Any way	2%
In no way at all	18%

With sugar	No sugar	Indifferent	In no way
74%	1%	8%	17%

8. Question -- If SNS would not distribute milk free, would you
buy it in the market?

Answer -- (In percentage)

Yes	No
56%	43%

9. Question - Why wouldn't you buy it?

Answer -- (In percentage)

Children don't take it	54%
I don't have the money	29%
I don't know	5%
Other answers	16%

10. Question - Why would you buy it?

Answer -- (In percentage)

Children put on weight with it	16%
It is very nourishing	52%
The children grow bigger	12%
They like it	16%
I don't know	4%

11. Question - How long does the package of "Leche Alim" last?

Answer - (In percentage)

One week	15%
Two weeks	12%
Three weeks	31%
Four weeks	40%
More than four weeks	2%

On the basis of this survey, it is quite obvious that the acceptance by the mothers of the Leche Alim formula is good, although some of the answers to the questions seem contradictory. Approximately 60% of the mothers say that their children like Leche Alim, and 40% say they don't like it. From the group that suggests that they do not like Leche Alim, 35% have given much too uniform an answer, in that they suggest that none of their children, none of the adults, or they themselves like it, and 5% say that some in the house don't like it. On the other hand, from the 40% of the mothers who say that their children don't like Leche Alim, only 18% of the 40% have discontinued obtaining it from the distribution center. This suggests that the rejection is only apparent and that it is caused by other factors. It is rather apparent that a very large percentage of these people wish

to revert to the use of powdered milk, and cross correlation indicates that they like the milk since it can also be used by the adults and thus would serve a much wider population base than the Leche Alim.

Sixty percent of the mothers who indicate that they like Leche Alim and are satisfied with the new product is quite remarkable on the basis that the product was not advertised or promoted in any form.

The percentage of protein that really reached the infant for whom the material is intended seems to be clearly higher than that of milk, since the Leche Alim package lasts close to four weeks, indicating that the child for whom the food was intended is the one who receives it. In earlier surveys we found that the milk lasts only six days in a normal family of five, indicating that it is being distributed among the entire family. This is not totally the case, however, since in many cases when the mother was asked if older children liked Leche Alim, the answer was "yes" in a high percentage of the cases. Apparently, since the traditional use of toasted wheat flour is for children, these were the major recipients.

Overall, it is also of extreme importance to note that 56% of the mothers who were interviewed say that they would buy the Leche Alim in the market if the National Health Service would not give it away free. This is certainly a high percentage when compared to many other surveys. On the other hand, the individuals who say that they would not buy it indicate in a significant percentage that they would not because they don't have the money.

These data coupled with the acceptance data of the extruded product would indicate that the significant advance in infant food production in the southern Latin-American region is possible by formulating products based upon indigenous protein sources. These will find moderate to excellent acceptability in the population and can be produced at significantly less cost than milk. They can have the equivalent nutritional characteristics to milk.

3. Detoxification of Rapeseed Meal

Work on the detoxification of rapeseed for animals has proceeded in a most satisfactory fashion. The rat studies cited before are now in the fourth generation. There is no evidence of toxicity from the detoxified rapeseed meal in the animals. All histology is considered normal; weight and growth patterns are normal. Reprints of the papers concerning the details of the initial work in this area have been submitted previously. Papers II and III on the detoxification process have now appeared in the Journal of Science of Food and Agriculture. The reprints are on file with our last summary report. A method for producing an isolate of non-toxic protein from rapeseed has been performed on a laboratory scale utilizing a salt extraction. This method yields a protein of high biological value compared to other protein isolates and produces a feed-grade protein as a byproduct. A preprint of this paper describing the methodology which apparently is patternable is attached. It has been accepted for publication in the Journal of Cereal Chemistry.

At the present time equipment for scaling the detoxified rapeseed has been purchased by the Institute of Food Science and Technology of the corporation for development and is being erected in Santiago. Pilot scale runs on the detoxification of rapeseed are to be initiated in September 1970, and it is anticipated that the production of this will be used in completing the toxicological studies in a second species of animals, in this case the miniature pig. Some studies in the miniature pig have already been initiated and the results indicate no substantial toxicological problems. However, in order to show that the detoxified meal is suitable for long-term feeding of non-ruminants, it is anticipated that a two-year study is required in pigs and a life-time study in poultry before wide-scale utilization of the detoxified meal can be anticipated.

4. Development of Other Protein Sources

Some work has been initiated on the production of the extrusion of high lysine corn and corn fortified with lysine. These studies suggest that an expanded product made from corn may very well find a place in the nutrition of Chile despite the fact that corn is not considered a product suitable for human consumption below the Tropic of Cancer in Latin America. By expanding the product the traditional corn character is altered sufficiently that the material does not resemble the corn-like product.

Experiments on the extrusion processing of fish-wheat or fish-cereal products have been discontinued on the basis that the expansion unit as it is now set up does not give a satisfactory cook or expansion. If these studies are to be pursued in the future, it will be necessary to modify the expander to allow a direct input of the protein raw material to the screw.

Papers Published or in Press

The following papers have been submitted for publication during the past period. They were presented at various meetings in Chile, and it is anticipated that they will be published in the Archives of Nutrition of Latin America in the near future. Reprints will be submitted when they are available.

- (1) "Extracción, Purificación y Valor Nutritivo de la Proteína de la Torta de Raps". D. Owen, C. Chichester, F. Monckeberg. Sociedad Chilena de Nutrición, Bromatología y Toxicología Reunión Anual Santiago 11-12 de Diciembre 1969.
- (2) Separación y Detoxificación de la Proteína de la Torta de Raps en Escala de Planta Piloto. D. F. Owen, C. O. Chichester, F. Monckeberg. Sociedad Latinoamericana de Investigación Pediátrica IX Reunión Anual Dec. 1, 2, 3, 1969, Valdivia, Chile.
- (3) Extracción de Proteínas de Torta de Raps para consumo Humano. D. Owen, C. Chichester, F. Monckeberg. Primeras Jornadas Anuales de Pediatría, Sociedad Chilena de Pediatría Nov. 26-29, 1969, Santiago, Chile.

The following paper has been accepted for publication in Cereal Chemistry and will appear in the October issue of that journal. A pre-print of the paper is included - "A Process for Producing Non-Toxic Rapeseed Protein Isolate and an Acceptable Feed By-Product", D. F. Owen, J. F. Monckeberg, C. O. Chichester.

Budget (current year)

	Appropriated	Obligated	Balance
Personnel	36,864.93	15,624.94	21,239.99
Supplies and equipment	14,112.89	8,393.02	5,719.87
Travel	5,187.68	1,498.56	3,689.12
Other	2,964.65	72.00	2,892.65

Table 1

Composition of Processed (Non-Fat) Leche Alim

Moisture	7.0%
Ash	4.7%
Fat	1.7%
Protein (min.)	26.8%
Fiber	1.9%
Available Lysine	6.5%
Carbohydrate	57.9%

Table 2

Effect of Processing on the Physical Characteristics of Leche Alim

	Consistency (cm)	Water Absorption Index	Water Solubility Index
Unprocessed	24	1.96	33.9
Processed	2.6	1.60	37.0

Table 3

Nitrogen Solubility of Leche Alim

	Protein Content	N Solubility (%) in H ₂ O	N Solubility (%) in 0.02N NaOH
Raw Mix	30.65	34.61	58.17
Full Processed Leche Alim	30.26	10.40	59.00

Table 4

Effect of Storage on 4% Fat Leche Alim

Lot #	neg Peroxide/ 1000 gm fat	Time of Storage at 25°C. in weeks
1	0.06	28
2	0.06	26
3	0	24

Table 5

P.E.R. of Processed Products at Optimum Conditions

	P.E.R.
Unprocessed Leche Alim	2.53
Processed Full Leche Alim	2.90
Processed wheat, sunflower, and FPC with milk solid added after expansion	2.91
Processed wheat, sunflower, with milk solids, FPC added after expansion	2.87
Casein control	2.50