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RESEARCH ANNUAL REPORT

"Development Program Directed Toward the Development of Infant Foods Based Upon Indigenous Proteins"

AID/csd - 3646

February 1973

REPORT SUMMARY

A. 1. Project Title and Contract Number:

Development Program Directed Toward the Development of Infant Foods Based Upon Indigenous Proteins

AID/csd-3646

2. Principal Investigator, Contractor and Mailing Address:

C. O. Chichester
Department Food and Resource Chemistry

University of Rhode Island
Kingston, Rhode Island 02881

3. Contract Period (as amended):

From June 30, 1972 to April 30, 1974

4. Period Covered by Report:

From June 30, 1972 to January 31, 1973

5. Total A.I.D. Funding of Contract to Date:

$28,951.00

6. Total Expenditures and Obligations Through Previous Contract Year:

$28,951.00

7. Total Expenditures and Obligations for Current Year:

$28,951.00

8. Estimated Expenditures for Next Contract Year:

$80,755.45

B. Narrative Summary of Accomplishments and Utilization:

The principal accomplishments of the period reported consist of the following:

1.) The testing of the product developed in previous years, that is Leche Alim, in a population. It was shown that this is readily acceptable to the population of the lower socio-economic levels of Chile and that in a twelve month testing period of supplemental feeding of pre-school children, significant increases in the heighth of children receiving Leche Alim were observed.

2.) The development of a secondary product, named Fortesan, which is an extruded instantly re-hydratable material based upon a wheat-soya blend.

3.) The training of Chileans in the area of communications.

These accomplishments in conjunction with those previously reported indicate that the team which has been developed in Chile is capable of undertaking product development, nutritional investigations, acceptability trials, and to develop marketing data.

Wider distribution of some of the products that have been developed is anticipated in the present contract.

Data on other protein sources has been reported previously but work on these protein sources has continued over the period of time in an effort to reduce the cost of the production of some of these materials.

GENERAL BACKGROUND

In Chile, as in most developing countries, one of the most serious medico-social problems is chronic infant malnutrition, retarding all development and growth and promoting low psychological performance and high susceptibility to infection. In Chilean families of low economic status, children in the first year of life receive the same food as the rest of the family. The protein and calories may be sufficient for adults, but not for the growing child. A high quality protein must be added to the diet, especially in the more vulnerable growth period.

Although a number of products have been developed in Chile which are eminently suitable for weanling foods, and foods for pre-school children, the commercial distribution of these materials is not what we believe it should be. Some of the foods are used in the national school lunch program and are produced commercially by a Chilean firm. Marketing of them however has not been extensive. This is due, in part, to the social organization of the country in which many of the food distribution programs are carried out either through the school lunch program or the National Health Service in child care centers, resulting in a minimal incentive to market the food materials commercially. The National Health Service has relied heavily upon powdered milk, a product which currently is in short supply in Chile and in moderately short supply in the world. This has resulted, in the last year, in large scale purchases of 12% fat powdered milk from the world market by the government of Chile. Prospects for continued importation of powdered milk are not good, and the cost will increase significantly in the next few years.

Sunflower meal is a particularly good source of the amino acid methionine. The combination, thus, of a legume protein with sunflower protein yields a combination which has an excellent nutritional value. Although the legumes (in this discussion excluding soy) are widely used in the Latin American diet, they are not generally considered as food for children or infants. This is probably due primarily to two problems. The first is that, in general, they are toxic in their raw state, and therefore, considerable preparation is involved before they can be consumed. Beans must be cooked for a long period of time, and in many cases pre-soaked. All the edible legumes

known contain hemaglutins and therefore must be subjected to heat treatment to destroy or to modify the protein. This is a problem, of course, in raw soybeans which, in addition like many other legumes, may contain trypsin inhibitors. All of these toxic materials are destroyed by heating so that, at least in adults, the products are normally consumed without difficulty. In the feeding of children, particularly at home and on demand, the problem of long cooking times is a major one.

In addition to these problems, the utilization of beans in young children presents some difficulty due to the problem of digestive upset caused by a decrease in passage time through the gut. Combined with this, beans may cause flatulence.

While the number of studies in experimental animals of the nutrient value of beans is extensive, observations on the biological value in humans are few. The legumes are rich in protein, and in fact, contain several times the protein content of cereal grains on a solids basis. Beans, as a representative legume, have a protein score which is decreased by the low content of the sulphur amino acids, particularly cystine and methionine. The legumes, however, also contain a significant amount of calcium and iron, as well as thiamine and nicotinic acid. The utilization of legume proteins in Latin America, paricularly the southern regions, is thus very attractive. Since the major nutritional shortage of the legumes is methionine, it would be economical to fortify with methionine; but an alternate is also attractive. In the southern regions of Latin America there is, as has been pointed out many times, a significant production of sunflower for oil.

It has recently been shown that the oligosaccharide stachyose is the major causative factor of flatulence in legumes. The elimination of stachyose by diastase treatment significantly reduces the flatulence effect of soy milk and presumably other leguminous products.

It is thus possible economically to produce products from the legumes which are easily prepared for consumption and contain no toxic or deleterious materials and produce a minimum of flatulence. These products, then, offer an excellent source of protein.

While these problems are of considerable significance in the production of children's or weanling foods they are not, by any means, insolvable. It is

quite possible to produce products which have been pre-treated to decrease cooking time required by a significant factor and this, in turn, will obviate any toxic factors. These same processes have the possibility of improving flavor and increasing the ways in which these products can be used in various formulated foods.

It is therefore important, both from the standpoint of health and economics, to increase the market and manufacture of the food materials which have been developed under this contract. Cooperation has been achieved between the National Health Service and our investigative team to develop an increased effort in the marketing, manufacture and distribution of two of our products - Leche Alim and Fortesan. It is thus possible to combine the efforts of marketing expertise available in Chile and the efforts of two private concerns interested in promoting the increased use of these food materials. It is obvious that cooperation of the National Health Service is urgently needed. This appears to be forthcoming at the present time.

Table 2 illustrates the essential amino acid content of various leguminous seeds. As pointed out before, these products are good sources of lysine but lack methionine and, in some cases, tryptophane. The tremendous world resources of the protein represented by the legumes require a great deal of additional work in order to effect their utilization. There is no question that they could serve as excellent sources for the preparation of protein concentrates or isolates.

The production of protein isolates and concentrates has proceeded at a moderately rapid rate in the developing countries. Generally, these products are utilized as animal protein replacers in formulated foods, although some have been sold as simulated meats. Processes for the production of this type of plant protein are comparatively expensive since they, in most cases, must serve a very specialized market where emphasis is placed upon their functionality in a food material. There are, however, a number of processes which can be utilized in the production of less sophisticated meat or animal protein simulants. An example is the structuring of proteins brought about by high-pressure extrusion techniques. This technology is of extremely low cost and produces an oriented fiber product. In Latin America, much of the animal protein that is consumed by the lower socio-economic classes is consumed in

the form of a soup or stew dish. Animal proteins are usually included as chunks or small pieces. By using a long barrel on a Wenger Expander Unit structural orientation of vegetable proteins can be achieved. In a series of experiments performed in the United States, it was possible to produce proteins from sunflower seed meal concentrates and sunflower protein isolates. These products would withstand cooking and could be tailored to various textural levels. If products of this sort could be introduced into the school lunch program, one would have direct utilization of vegetable proteins in a traditional dish used in the entire Latin American region. Costs of such a product would be considerably lower than those of the animal proteins which they will replace. Additionally, since the products are textured the fortification of the plant protein would be comparatively easy, and no problems with regard to leaching would be encountered. A structured protein of good balance could be developed utilizing legume protein in conjunction with sunflower, or alternatively, combinations of fish protein concentrate, or fish protein with other plant proteins. Previous experience would indicate that appropriate flavors could be incorporated into the products at low cost.

STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT

It is believed that the group of institutions, that is the Department of Pediatric Nutrition of the University of Chile, the marketing group of the Catholic University, the Institute for Science and Technology of Chile, the Institute for the Development of Fisheries and the Department of Food Science and Technology of the University of Chile, together with the Department of Food and Resource Chemistry and the Department of Resource Economics of the University of Rhode Island, offer a combination of facilities, personnel, and enthusiasm which is difficult to match elsewhere. Since the project proposed is a broad-based one involving carrying products from the laboratory phases of experimentation through pilot scale production acceptability testing and marketing each unit serves a particular function and each contributes to the overall project. The coordination of all of these efforts has resulted in significant advances in the nutrition of pre-school children in the Chilean region and has, because of its success, developed a great interest and support at governmental levels. It is thus important to continue work in this line for a period sufficient to assure the establishment of commercial production and marketing of the food developed. Each unit is able to work effectively with the others and to make its contribution. One could not dispense with technology, nutrition, toxicology or consumer acceptance testing, etc. The United States base makes its important contribution by training personnel from the various institutions in the host country, by coordination, and by backstopping the research effort with facilities, equipment and expertise in the various fields.

The specific aims outlined next would be attacked more or less simultaneously, but a major effort will be placed upon the marketing aspects of already developed foods.

1. The development of marketing information and a subsequent marketing promotion of the products which have been developed previously, e.g. Leche Alim and Fortesan.

This program will require liaison between the Catholic University (School of Economics), the University of Rhode Island (Department of Resource Economics), and the groups in INTEC. These groups will also be used to develop

the marketing programs for other products produced under the current contract.

Cooperating in market development, because of the nature of the Chilean social situation, will be the National Health Service and the National School Lunch Feeding Program. We have already had cooperation from these groups and they have indicated their willingness to continue work for the actual product marketing aspects of the products. Integrated with these groups will be two private enterprises in Chile and the international group of Nestles. They have already packaged numerous samples for the acceptability trials in Curico and will participate in the marketing aspects of the present study.

2. The development of high protein, low cost foods based on the utilization of legumes in conjunction with other vegetable protein sources such as sunflower meal.

These products would be processed in order to obtain a readily rehydratable product with no toxicity utilizing expansion techniques. In this area, we would also attempt to develop a series of structured proteins of inherent low cost based on supplemented legumes which could be used in traditional Latin American dishes.

Toxicity and nutritional testing would be done at the Department of Pediatric Nutrition of the University of Chile, and in the Department of Food and Resource Chemistry at the University of Rhode Island in the United States. Enzyme studies would be conducted at the Institute for the Development of Fisheries and pilot scale processing would be performed at the Institute for Science and Technology in Chile.

3. The development of low cost co-precipitants of cereal of legume protein with marine proteins.

A series of products which would utilize marine proteins to supplement the deficiencies in the amino acid profile of cereal proteins would be developed. These would be processed in the same fashion as those developed in Aim #1 so that the products would not be a mere mixture of two different protein sources but rather an integral protein of mixed origin. The work on nutritional testing of these products would be performed in the Department of Pediatric

Nutrition of the University of Chile and in the United States. Process development would be done at the Institute for the Development of Fisheries and the Institute of Science and Technology in Chile.

4. The training of Chilean nationals in protein processing problems, and market development.

This training would be conducted in the United States and initially would focus on the training of an individual in the analysis of microtoxins, a skill which is badly needed in Chile and for which no personnel are now available. The second area of consideration would be the study of methods of producing structure proteins. A third need is for training in the area of market development.

CONTINUED RELEVANCE OF THE OBJECTIVES

The project has developed logically over the past. The number of food materials and processes have been outlined and thus in the present portion of the contract, the objectives are determined with some assurance of the marketability of these products.

The objectives have been modified to some extent to allow for the investigation of protein sources other than those from oilseeds and work in this area has been initiated. Additionally, the development of meat substitutes utilizing expansion techniques are part of the modified objectives of this program. Thus, the aim of the present contract is in line with the rational development of a long term program.

ACCOMPLISHMENTS TO DATE

The project (AID/csd-3646) has been underway for a number of years concerned with the development of infant and weanling foods based upon oilseed proteins and fish protein concentrate, which are available in the southern Latin American region. This project has utilized protein sources hitherto not utilized for the production of these foods. The project is an integrated one which investigated possible food protein sources in a systematic manner. The protein sources were evaluated nutritionally by both chemical and biological means, and toxicological investigations of the foods were undertaken in two species of animals. The technology of formulation was developed at a laboratory scale, acceptability trials of the formulated foods were made, and the technology for larger scale production was worked out, along with the economic evaluations of the process. Finally, the products were produced on a commercial scale and evaluated in target populations. The sources of food which were initially considered in this project were fish protein concentrate and sunflower meal presscake. Later, rapeseed presscake meal was added to the products that were investigated. At the present time the sunflower meal presscake is being utilized commerically on a moderate scale and the technology of detoxification of rapeseed meal by commercial scale is being developed. The work on rapeseed meal has attracted considerable attention world wide.

A third food material has been developed in the last year based upon a specially processed wheat and soy base (wsb) to which other nutrients are added to give an instantly rehydratable weanling food of very high biological value.

The chief achievements of the project outside of the scientific achievements listed can be considered in three areas, as follows:

1.) The establishment of an integrated program in applied nutrition which may serve as a model for other areas of the world. This integration has been achieved by training of Chilean and other nationals and the development of linkages between the basic groups and the applied technological groups in foods in Chile. The program has been able to develop a central applied nutrition theme through the efforts of two governmental agencies, four universities and

two business organizations, all of which are pointed to a common aim.

2.) The development of national programs for the supplementation of infant diets through the development of food products based upon indigenous protein sources that are targeted at the most vulnerable groups in the Latin populations, that is, the pre-school infants.

3.) A research team has been developed in the country which has achieved some very significant results which may be applied world wide. The results of the relationship between mental development and malnutrition in early youth, the demonstration that marine protein concentrate can be utilized in infant feeding successfully, and the development of new protein sources from underutilized products such as sunflower seed presscake and rapeseed presscake are significant research breakthroughs.

The results of this contract contribute to institution building, the development of interactions between research groups and private industry in Chile, and obviously, to the development of research results which are significant on a world wide basis.

The overall project involved the Institute of Food Science and Technology of the University of Chile, the Institute for Science and Technology of the Development Corporation of Chile, and the Institute for Fisheries Development (their section on food technology) together with the Catholic University of Santiago. The project in Chile was lead by Dr. Fernando Monckeberg of the Department of Pediatric Nutrition of the University of Chile. These institutions worked together on the overall project, each in its separate area of expertise.

The National Health Service of Chile contributed manpower in the portions of the project concerned with the field testing of products on a large scale.

Coordination of the efforts in Chile and the training of individuals from these institutions was undertaken by the United States institution. Portions of the nutritional and chemical analyses were done in the United States and personnel from the United States worked in Chile between the various institutions on some of the processing and nutritional phases.

It is proposed that the present project employ the same groups since it has been found that, by coordinating the efforts of these institutions and by cooperating with interested commercial groups, significant progress can

be made at a fairly rapid rate. Additionally, the inputs of each of these segments are synergistic. The lead group in Chile is the Department of Pediatric Nutrition of the University of Chile and, in the United States, the Department of Food and Resource Chemistry at the University of Rhode Island. In order to promote the regionality in the program, liaison is maintained between the University of Buenos Aires, Department of Nutritional Sciences; the Department of Food Science and Technology of La Molina in Peru, and the Institute of Food Technology Research in Brazil.

Under AID/csd-3646, approximately 32 papers have been published or presented. Appendix I shows the papers that have been presented at international meetings or published during the last two years of the contract.

INTERPRETATION OF DATA AND SUPPORTING EVIDENCE

Appendix 3 includes a "Preliminary Report on a New Protein-Rich Mixture Called Fortesan" and an excerpt from the Project Semi-Annual Report (September 1970) on the Acceptability of Milk Substitute in Curico.

RESEARCH DESIGN

No significant changes in research protocol have been incorporated in the report period.

DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Appendix 1 present a list of publications relevant to this project. These publications have been used to disseminate information developed in this work to the scientific and health related communities.

Included within this list are speeches presented at conferences, seminars, etc.

The publications listed have been widely reported in both Spanish and English versions.

It is not possible to give the citations of all the many papers related to this project. Needless to say with the large number of publications, speeches, etc., the group in Chile has developed a world wide reputation on the basis of their work and consequently uses of this material have been so widely reported that it is impossible to keep track of it all.

2. There are at least three projects in this work on the detoxification of rapeseed. This is being utilized on a fairly large scale in Argentina. Additionally some of the Canadian groups working in the detoxification of rapeseed have modified to some extent the techniques reported from the work in Chile.

A patented application has been made based upon the technique for isolating non-toxic protein isolates from rapeseed.

As modifications of the work are reported, some industrial work is occurring in the United States on the production of protein isolates using the rapeseed technique developed for rapeseed isolation.

3. Wide dissemination of results through publication to the health and nutritional communities have proven rather effective. Coupled with the utilization of reports in food technology literature, this methodology of dissemination has proven effective.

The presentations at numerous meetings of project data is also of significance since it encourages direct discussion with the speakers and interested parties in the audience.

The utilization of mass media within the Latin American region is also effective. This aspect of the project could be expanded significantly however. Reports of the development of food materials and research results in nutrition to the lay public may significantly add to their nutritional awareness. This is a task which needs to be carried out but would have to be carried out under a separate contract.

4. The great majority of this work is done by the individuals in Latin America. The function of the University of Rhode Island and the principal investigator is primarily that of backstopping.

The personnel in Chile have of course been intimately involved in the development of the project, analysis of the data and the execution of the work.

STATEMENT OF EXPENDITURES

The following is a budget break-down for the project period from June 30, 1972 through January 31, 1973.

Salaries and Wages	
U.S. Personnel	5,545.10
Cooperating Country National	12,570.60
Fringe Benefits	621.05
Indirect Costs	2,173.68
Consultants	0
Travel and Transportation	0
Equipment and Supplies	8,040.57
TOTAL	$ 28,951.00

WORK PLAN

I. Legume Utilization

The operating parameters for pre-cooking Chilean black beans and other legumes in a Wenger expander will be determined. A pre-conditioning and, possibly, a pre-cook step will be used to lower toxicity. The resultant product will be ground to a mash which will give ready reconstituteability. The toxicity as well as the nutritional value will be determined using rats as the test subjects. The product will be compounded with other proteins or fortified.to give a requisite FAO pattern. The moisture of sunflower seed and beans will be used in soup base dishes for which a tradition exists. Acceptability of the mix will be determined in restricted populations (orphanages) in Santiago, using children aged 3-7. The nutritional effectiveness in normal children will be determined using previously described techniques.

. Bean slurries will be digested with diastase in order to decrease the stachyose content of the product or processed in a long barrel extruder. The degree of removal of stachyose will be determined chemically, and the flatulence level of the product determined by the method of Criseofareo et al. in adult male rats. If sufficient reduction in stachyose content is achieved, the product will be tested utilizing normal infants.

The method of sprouting prior to cooking and drying to achieve stachyose and raffinose reduction will be compared against the diastase methodology. The economics of both methods of oligosaccharide reduction will be determined.

An expanded barrel for the Wenger X-25 expander will be purchased and installed in Chile and an X-5 for laboratory investigations will be installed in the United States for training and production modification. Training of an individual from INTEC on the use of the equipment will be undertaken in the United States industrial laboratories. The feasibility of producing structured corn, sunflower, and rapeseed proteins will be determined (these are all indigenous to Chile). The products or mixtures of these products will be assayed for nutritional value and acceptability in limited trials. Formulations utilizing these structured proteins in the typical soup or stew dishes will be made. These in turn will be assayed for acceptability

in school lunch programs in the Curico area south of Santiago. Cooperation in the development of these meat substitutes will be maintained between the industrial segment in Chile and, since the processing work will be done under the auspices of the Cooperation for Development, it is likely that if the products are accetable in the Chilean dietary, their translation into commercial channels will be facilitated. It is likely that they will find a place in the national distribution of lunch programs since at the present time the distribution by the Ministry of Education of luncheon breakfasts approximates $15,000,000 a year in Chile. A significant percentage of this cost involves the use of animal protein, a major portion of which is imported.

Listed below are a number of controllable variables for the extrusion process which control the nutritional qualities and acceptability of products produced. Although these could represent a myriad of experiments, based upon previous research, we can narrow the range of conditions which we will investigate, in other words, each of the variables will not have to be investigated independently.

Material types	Material variables	Equipment variables
Legumes	Moisture content	Temperature
Wheat flour	Particle size	Shear rate
Soy flour	Additives	Die configuration
Rapeseed protein	Conditioners	Feed rate
Sunflower protein	Colors	Barrel pressure
Vegetable oils	Flavors	Die pressure
Fish oils	Amino acid fortification	Output rate
Sugars	Homogeneity of raw mix	Secondary drying conditions
Dried skim milk		
Fish protein concentrate		

In total, however, we will investigate three basic methods of processing the proteins as follows:

Low pressure cold forming

Low pressure cooking and forming

High pressure cooking and forming

In all cases, we will attempt to achieve functional characteristics and apparent characteristics similar to meat by this method which has been very

successful when applied to soy proteins. In particular, the products should have the following characteristics:

1. Low cost
2. Good nutritional value
3. Texture similar to meat
4. Meat-like flavor and color
5. Ability to withstand cooking in a typical Chilean dish

II. Development of Protein Co-Percipitants

In the processing of expanded or structured proteins, mixtures of various protein sources (such as milk, sunflower, bean, etc.) can be added to the expander unit. These result in an intimate mixture of the protein sources in such a manner that, even after grinding, the product is not segregatable. Further processing such as drying, coating, etc. results in a more or less homogeneous mixture of protein sources. Thus, in the expanded mixtures it is possible to develop a single protein base. The acceptability trials of these intimate mixtures using different processing parameters will be performed to determine within nutritional limits the processing techniques which result in developing the most desirable characteristics. Such techniques as oil dipping, spray addition of flavors, utilization of incapsulated flavors, toasting, etc., will be investigated.

A technique which will also be considered in this area is the actual co-precipitation of proteins. In previous work we have been able to develop a fish protein concentrate which is soluble at high pH's and is precipitable at its isoelectric point, e.g., approximately 4. Simultaneously, in working with rapeseed and sunflower, protein isolates using either salt extraction or high pH solubilization have been produced. The next logical step which will be investigated in this phase of the contract will be the precipitation of mixed proteins at pH's 2 to 4. This technique should result in a protein isolate which has high nutritional value (better than casein) that can be utilized in other food products as a non-separating protein source. Co-precipitants with the addition of flavoring components and other constituents such as hydrogenated fat will be investigated as to acceptability and

nutritional effectiveness.

III. Training of Chilean Nationals

The individuals will be brought from Chile to universities in the United States or other institutions where they can obtain training. The stipend in the United States portion of the budget will cover costs incurred while they are here. As a first order, the training will be given for a short period of time to an individual who will be able to determine the aflatoxin or microtoxin and pesticide contents of raw materials that go into the various food formulations and also that of the finished product. These and the other training areas outlined under the specific aims will be facilitated by cooperation of the United States institution with other institutions in the United States and with government and private sectors.

IV. Marketing

It is apparent that when one seeks to introduce a new product to the market, background information on that market, such as consumer purchasing power, purchasing habits, geographic distribution, size, distribution of retailers and retailing purchasing habits are needed in order to develop general models for the diffusion of innovation to the particular market. This was city by Wickström in Protein-Enriched Cereal Foods for World Needs (edited by M. Milner). In particular, we will have to determine the marketing instruments that in previous efforts have been shown to have the best effect. This information will not have to be generated de novo since the Catholic University has been studying marketing of food products in Chile for some time and there is available to us the marketing survey done in recent years by General Oceanography. Much of this information can be utilized in the marketing survey and subsequently in the market promotion of Leche Alim and Fortesan, or for that matter, all other types of new food products.

Additional information is available due to the development of markets for protein rich foods, such as Incaparina in Central America, Pro-Nutra in South Africa, Vitasoy in Hong Kong, etc. Case history studies on the marketing of these products (N. Wilkie, Marketing Strategy in Developing Countries to Combat Infant Malnutrition, Third International Congress of

of Food Science and Technology) will aid in the development of marketing strategy for the products developed in Chile. We will develop specialized data for Fortesan and Leche Alim by presenting the pilot plant samples of the products to respondents in differnt Chilean cities to determine:

1) Concept evaluation
   a. CRAM scores
   b. key benefits
   c. product uniqueness
   d. product negatives
2) Product evaluation
   a. specific product attributes, such as flavor, texture, consistency, color, etc.
   b. key benefits
   c. problem areas - negative attribute analysis
   d. preparation
   e. serving
   f. possible frequency of use
   g. likes and dislikes
   h. interest in purchasing
   i. the possible frequency of purchasing
   j. price/demand structure
   k. packaging

These tests consist of two phases: first, a screening/concept/placement interview and secondly, a follow-up interview. In phase one, the families are to be screened for the presence of children. Those qualifying will be exposed to concept advertisement and their reaction to it explored. Phase two involves interviews which will cover the product performance and reaction of the family having been presented with the products themselves. Thus, primary information on acceptability of the product in the market place can be learned and the perspective volume of the available market can be estimated.

Having determined effective marketing information, the distribution channels, be they government or private, can be utilized to launch a limited test of marketing. We will probably use the city of Curico which has been exposed to the products previously. This exercise will provide a method of estimating the real demand at a given price level and at a given promotional

level. Once the given optimal feedback from the limited test marketing is established, the possibilities for promotion campaigns for national marketing distribution can be reviewed with the interested private concerns in order to illustrate to them the possible marketing opportunities for such foods. In particular, they are desirous of ascertaining the promotional level which will be needed to develop the market outside of the national feeding organizations.

Within the school lunch program and the National Health Service, there is a desire to utilize these products. This will be necessary in order to develop a base production. We will use the results of our marketing development to convince them of consumer demand. This coupled with the promotional efforts and placement trials should develop an internal demand for the products, and thus in turn stabilize the base of production. National television and radio have established a campaign for nutritional education of the public. Groups in Chile have obtained assurances that a portion of this time can be utilized for the introduction of new products, thus in effect, the Chilean government will assist in the market development of the products.

BUDGET FORECAST FOR THE COMING YEAR

Proposed Budget for time period from February 1, 1973 through April 1, 1974

Salary and Wages		
U.S. Personnel		
professional	9,800.00	
non-professional	3,675.00	
		13,475.00
Cooperating Country Nationals		
professional	17,640.00	
non-professional	8,758.00	
		26,398.00
Fringe Benefits		992.25
Consultants		1,500.00
Travel and Transportation		
United States	608.00	
International	4,200.00	
		4,808.00
Equipment and Supplies		
Expander and parts	21,500.00	
Commodities, chemicals and laboratory supplies	5,800.00	
Freight	1,000.00	
		28,300.00
Overhead		5,282.20
TOTAL		80,755.45

TABLE 1

Amino Acid Content of Several Oil Seed Proteins
(g/16 g N)

Amino acid	Rape (*B. napus*)	Soybean meal	Sunflower meal	Cottonseed meal	Peanut meal
Lysine	5.8	6.5	3.5	3.9	3.0
Methionine	1.8	1.8	2.2	1.7	1.1
Cystine	2.4	1.7	1.8	1.0	1.4
Leucine	6.3	8.1	6.9	6.7	7.6
Isoleucine	3.6	5.0	4.2	3.9	3.8
Valine	4.8	5.1	5.8	6.0	4.7
Phenylalanine	3.5	4.8	5.1	6.3	5.2
Tryptophan	---	1.5	1.4	1.3	1.0
Threonine	3.8	3.7	3.4	3.6	2.0
Arginine	5.6	8.3	9.1	7.4	11.6
Histidine	2.6	3.3	2.8	2.5	2.5

TABLE 2

Amino Acid Content of Pulse Proteins
(gm/16gm N)

Amino acid	Chick pea	Pea	Broad bean	Lentils	Common bean
Arginine	7.45	8.83	7.00	7.63	6.02
Histidine	2.68	2.74	2.94	2.19	2.84
Isoleucine	5.74	5.63	6.27	5.26	5.68
Leucine	7.39	8.27	8.70	7.04	8.59
Lysine	6.896	7.33	5.62	6.11	7.42
Methionine	2.75	2.50	1.12	1:54	2.00
Phenylalanine	8.19	9.07	6.86	7.07	9.37
Threonine	3.55	3.86	3.26	3.58	4.34
Tryptophan	.82	1.06	.93	.86	.93
Valine	4.93	5.60	5.02	5.44	6.06

APPENDIX 1

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A PROTEIN-RICH MIXTURE BASED ON DRY SKIM MILK, TOASTED WHEAT FLOUR, FISH FLOUR AND SUNFLOWER MEAL. Enrique Yanez, D. Ballester, C. O. Chichester and F. Monckeberg, University of Chile, Santiago, and University of Rhode Island, Kingston.

One of the most challenging problems faced by developing countries like Chile is that they are unable to produce the necessary food for their fast growing populations. This situation affects mainly the young child due to his high protein and calorie requirements and results in stunted physical and intellectual development.

As a means for alleviating the deficit of high quality protein in the diets of children, we have developed a protein-rich mixture called LECHE ALIM intended to be used as a gruel at breakfast or tea-time. This mixture contains: toasted wheat flour, 40%; dried skim milk, 40%; fish flour, 10%; and sunflower presscake meal, 10%.

Chemical analysis showed that Leche-Alim contains: protein, 27%; fat, 6%; and carbohydrates, 58%. To measure the biological quality of the protein, different indexes were determined: PER 2.56, NPU 72, Chemical score 74 and NDpCal% 14.7, all of them indicating a good quality protein.

In order to test the acceptability in a long-term program, Leche-Alim was prepared in a local industry and packaged in plastic bags containing 1.5 kg. As subjects, 1100 children from 2 to 5 years of age, living in both urban and rural areas of one of our provinces, were selected. Children under 2 years of age continued receiving their powdered milk as usual. All subjects were weighed and measured once a month.

At 3 and 12 months, acceptability was found satisfactory but lower than that of powdered milk containing 12% fat, and similar to that of dried skim milk. After 12 months of the experiment, the weight increase was 2.19 kg for children receiving Leche-Alim and 2.17 kg for those receiving dried skim milk. The change in height was 8.78 cm and 6.04 cm for children given Leche-Alim and milk respectively (P 0.001).

DETOXIFICATION OF RAPESEED MEAL. Digna Ballester, E. Yanez, M. Rojas, C. O. Chichester and F. Monckeberg, Universidad de Chile, Santiago.

We have reported elsewhere the feasibility of detoxifying rapeseed meal by water extraction for 14 hours and obtaining a material of high quality protein (NPU = 68). The present work was done in order to shorten the extraction time of toxic factors, isothiocyanates (ITC) and vinylthiocoxazolidones (VTO) thinking of an eventual industrialization of the process. The procedure now employed is a continuous water extraction with agitation at room temperature. A 30 minute extraction caused a reduction in the VTO and ITC content similar to that obtained with the method previously proposed, reaching a complete elimination of ITC after 2 hours.

The following table summarizes the results:

Treatment	NPU	Isothiocyanates ITC (mg/gm)	Vinylthiocoxazolidones VTO (mg/gm)
None	39	1.48	7.52
Maceration in water (14 hours)	68	0.37	1.32
Continuous water extraction:			
30 min	69	0.20	0.97
2 hr	70	0	0.16

The toxicity assay performed in rats with the detoxified material at both 30 minutes and 3 hours showed that these products have a growth promoting capacity very close to that of casein in contrast to untreated rape that showed a notorious inhibition.

The weights of the selected organs of the animals given treated rape were normal, with the exception of liver, which showed a slight increase. These animals showed no histological changes in the liver; however, a great majority of them presented histological signs of thyroid hyperactivity of varying degrees. The rest of the studied organs showed no histological differences from the control group fed casein.

From these data it may be concluded that the shortened and continuous treatment proposed for the rapeseed meal produces a greater reduction of toxic substances than the original one, resulting in a material the toxic properties of which have been reduced significantly and that could be used extensively in animal consumption.

SOME NUTRITIONAL CONSEQUENCES OF THE MAILLARD REACTION. **V. C. Sgarbieri, M. Tanaka, C. O. Chichester and J. Amaya, University of Rhode Island and Institute for Food Research, Campinas, Brazil.**

The Maillard reaction between free amino acids and reducing sugars yields several types of compounds (Hodge, J. E., *J Agr Food Chem* 1:928, 1953) which may have a detrimental effect on the nutritional value of foods undergoing this reaction.

In a series of experiments we incorporated browned and non-browned freeze-dried banana powder in a rat diet otherwise normal but low in protein (8% casein). The banana powder was the only source of carbohydrate in the experimental diets. The PER for the fresh banana diet was lower than the standard casein (2.5), but that of the diet containing browned banana was significantly lower than that containing fresh banana. As the contribution of banana to the amino acid pattern of the diet was low, the marked decrease in the PER of the diet containing browned banana is thought to be due to a low level of toxicity induced by the browning reaction.

In other experiments a completely synthetic diet was used. A mixture of amino acids in the proportion recommended for rats (Rogers and Harper, *J Nutr* 87:272 and 267, 1965) was incubated with glucose for 30 days at 37°C at a water content of 16%. At the end of the incubation period the amino acids were analyzed to estimate the losses, and feeding experiments with weanling male rats were performed. Complete supplementation of diets with the essential amino acids lost during browning estimated on the basis of chemical analysis, did not restore the nutritive value to the same level as the non-browned diet.

The calculated nitrogen efficiency ratio (NER) was significantly lower than the control in the browned diet and the supplemental amino acid browned diet.

The Amadori rearrangement product of two essential amino acids, i.e., leucine and tryptophan, was synthesized to determine its nutritional value.

Absorption studies were conducted by stomach intubation of the radioactive product in water solution. The availability of the amino acids in this form to the microorganism *Streptococcus zymogenes* and to the rats was also determined by growth studies. Our results indicate very poor absorption of the amino acids which had participated in the initial phases of the Maillard reaction, but an adaptive effect was evident in time.

RAPESEED MEAL

II.*—Chemical composition and biological quality of the protein

By DIGNA BALLESTER,† R. RODRIGO,† J. NAKOUZI,† C. O. CHICHESTER, E. YÁÑEZ** and F. MÖNCKEBERG**

The chemical composition (moisture, ash, protein, amino acids, ether extract, crude fibre, Ca, P, Fe, thioglucosides, isothiocyanates and oxazolidinethiones) and biological quality (net protein utilisation, available lysine and digestibility) were investigated in 12 samples of presscake meal of rapeseed (*Brassica napus*) collected from local factories. Crude fibre and mineral contents were comparatively high. Thioglucosides and their hydrolysis products (isothiocyanates and oxazolidinethiones, which are toxic) were found in high concentrations. All samples showed a high protein content and their amino acid patterns corresponded to a good-quality protein with a score of 80, and an adequate level of available lysine. Nevertheless, the biological quality measured as net protein utilisation and digestibility gave low values, which could be explained on the basis of the presence of the toxic factors and the high level of crude fibre, respectively.

Introduction

The cultivation of rapeseed (*Brassica napus*) as a source of edible oil has spread to many areas of the world. In Chile, its cultivation has increased markedly during recent years, reaching 87,000 tons a year,[1] and has made its use the largest among oil-bearing seeds. In addition to its importance as an oil-seed it is a potential source of protein, because after oil extraction a presscake is left that contains about 35% protein. However, before this material can be used extensively it will be necessary to solve the problem posed by the presence of goitrogenic[2] and growth-inhibitory factors[3] which are thioglucosidic in nature.[4,5]

This paper deals with the chemical composition of rapeseed presscake meal, with emphasis on the concentration of thioglucosides and the products of their hydrolysis, isothiocyanates [3-butenyl isothiocyanate] and oxazolidinethiones [L-5-vinyl-2-oxazolidinethione] (VTO), and its biological quality measured as net protein utilisation, available lysine, amino acid content and digestibility.

Experimental

Materials

12 samples of solvent-extracted rapeseed presscake meal obtained from local industries (A, B, C), and produced on different dates (1, 2, 3, 4) were used for chemical analysis.

Chemical methods

Water, total ash, calcium, phosphorus, iron, ether extract and crude fibre were determined according to A.O.A.C. methods.[6]

Nitrogen content was determined by the Kjeldahl method using a macrodigestion procedure,[6] followed by a semi-micro distillation in a Markham apparatus. The ammonia produced was distilled into 2% boric acid containing a mixed indicator.[7]

Amino acid analysis was performed by microbiological methods[8] from a pooling of samples from one of the industries (B). About 0·5 g of protein was refluxed with 60 ml of 20% by vol. HCl for 8 h (2 h for cystine).

Calorie content was calculated by using the factors 4, 9, 4 of Atwater.

Available lysine was determined using the 2,4-dinitrofluorobenzene (DNFB) procedure of Carpenter.[9]

Thioglucosides were determined by the procedure of McGhee *et al.*[10]

Isothiocyanates (3-butenyl isothiocyanate) and oxazolidinethiones [L-5-vinyl-2-oxazolidinethione] (VTO) were measured by the enzymic method described by Appelqvist & Josefsson.[11]

Biological measurements

The samples of each industry were pooled in order to measure their biological quality.

Net protein utilisation (NPU) was determined in 31-day old rats according to the method of Miller & Bender.[12]

Nitrogen digestibility was measured in the same experiment using the formula:

$$\text{True nitrogen digestibility} = \frac{I-(F-F_k)}{I} \times 100$$

where I = dietary nitrogen intake of the animals on the experimental diet, F = faecal nitrogen on the test animals and F_k = faecal nitrogen of the animals on the protein-free diet.

Results

Chemical analysis

The results of the chemical analyses are given in Tables I and II. In general the values show only a slight variation in the chemical composition, which reveals that the processing and seed varieties used by the three industries were similar. Only minor differences in the moisture and fat content were observed in samples of industry B. The protein, crude fibre, and ash contents were comparatively high and were similar in all samples. The calorie content ranged from 305 to 317 kcal/100g.

In general, the thioglucosides varied from 2·65 to 3·30%, the lowest being those of B. The isothiocyanate content showed a mean value of 1·4 mg/g and the VTO content was 7·6 mg/g. A good stoicheiometric correlation was observed

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TABLE I

Gross chemical composition and calorie content of rapeseed presscake meal obtained from three different factories and produced on different dates

Origin	Moisture, %	Ash, %	Protein (N × 6·25)	Ether extract, g/100g	Crude fibre, g/100g	Ca, mg	P, mg	Fe, mg	N-free extract*	Calorie content, kcal/100 g
Industry A:										
A–1	5·4	6·0	34·8	3·8	14·0	667	1050	15	36·0	317
A–2	5·3	6·2	34·0	2·5	14·0	658	1048	15	38·0	310
A–3	5·7	5·9	35·0	3·0	14·1	670	1042	14	36·3	312
A–4	5·3	6·3	34·4	2·5	14·7	669	1052	15	36·8	307
Mean	5·4	6·1	34·6	3·0	14·2	666	1048	15	36·7	312
Industry B:										
B–1	9·9	6·1	33·4	1·7	14·1	669	1068	14	34·8	310
B–2	9·6	6·0	31·2	1·6	13·9	672	1053	15	37·7	312
B–3	8·8	6·1	33·7	1·9	13·8	670	1071	14	35·7	313
B–4	9·2	6·3	34·1	2·0	13·9	677	1075	14	34·5	305
Mean	9·4	6·1	33·1	1·8	13·9	672	1067	14	35·7	310
Industry C:										
C–1	6·9	5·8	34·1	2·8	13·4	648	943	17	37·0	310
C–2	6·7	5·8	34·2	3·2	13·5	652	947	18	36·6	312
C–3	6·6	6·0	33·4	3·3	13·2	640	952	17	37·5	313
C–4	6·8	6·6	34·5	3·0	14·1	650	952	16	35·0	305
Mean	6·8	6·0	34·0	3·1	13·6	648	949	17	36·5	310

* Calculated by difference

TABLE II

Thioglucosides, isothiocyanates [3-butenyl isothiocyanate] and oxazolidinethiones [L-5-vinyl-2-oxazolidinethione] of different samples of meal

Origin	Thioglucosides,[a] g/100g	Isothiocyanates,[b] mg/g	Oxazolidinethiones,[c] mg/g
Industry A:			
A–1	3·12	1·51	7·93
A–2	3·09	1·52	7·82
A–3	3·05	1·48	7·79
A–4	3·10	1·49	7·86
Mean	3·09	1·50	7·88
Industry B:			
B–1	2·67	1·38	6·58
B–2	2·65	1·36	6·57
B–3	2·69	1·39	6·64
B–4	2·70	1·38	6·70
Mean	2·68	1·38	6·62
Industry C:			
C–1	3·30	1·36	8·39
C–2	3·15	1·33	8·27
C–3	3·18	1·30	8·32
C–4	3·28	1·35	8·35
Mean	3·23	1·33	8·33

[a] 2(R)-hydroxy-3-butenyl sodium glucosinolate
[b] 3-butenyl isothiocyanate
[c] L-5-vinyl-2-oxazolidinethione

between the content of thioglucosides and their hydrolysis products. The difference between isothiocyanate and VTO was due to the fact that only the former is volatile.

The values for different amino acids are presented in Table III.

Biological tests

The *NPU*, true digestibility and available lysine values are presented in Table IV. *NPU* and true digestibility results showed that the net quality of the protein was poor in spite of its apparent score as calculated on the basis of its amino acid composition. Nevertheless, all samples showed an acceptable level of available lysine. Differences in the above levels between samples were fairly small.

Discussion

The chemical composition of the rapeseed presscake meal was very similar in all the samples analysed, a predictable finding since the raw materials and the process were the same in every case.

The high protein content of this meal is in agreement with values reported by other authors,[13,14] and thus this product represents a large potential source of protein.

The high fibre content found in all samples could be an unfavourable factor in its future utilisation. On the other hand, rapeseed meal presscake is a good source of minerals such as Ca, Fe and P.

The thioglucoside content, even in small quantities, is important since it is related to the toxic effects observed in experimental animals fed this product.[4] It is known that isothiocyanate and VTO are released by enzymic hydrolysis, the latter being directly responsible for the toxicity of rapeseed presscake meal.[5] The values found for these substances agree with those reported in the literature.[15–17]

The values of the essential amino acid revealed a pattern resembling the protein quality of casein, with its limiting amino acid being methionine. This finding closely agrees with those reported by Clandinin and other workers.[5] However, this was not confirmed by the biological tests. This could be explained by a lower availablity of amino acids such as methionine and/or the presence of the above-mentioned substances.

TABLE III
Partial amino acid content of meal*

Amino acid	g/16g N	Organism
Isoleucine	3·6	*Leuconostoc mesenteroides* P60
Leucine	5·9	*Streptococcus faecalis* 9790
Lysine	4·8	*Leuconostoc mesenteroides* P60
Phenylalanine	3·3	" "
Cystine	1·6	" "
Methionine	1·8	" "
Threonine	3·7	*Streptococcus faecalis* 9790
Valine	5·9	" "
Histidine	2·1	*Leuconostoc mesenteroides* P60
Arginine	7·2	" "

* Tryptophan was not measured

Bell and other workers[2,3] have demonstrated that isothiocyanate and VTO inhibit growth and cause a clear goitrogenic effect. In addition, Ballester *et al.*[18] found a toxic effect on the liver of rats fed a diet containing 20% protein calories from a similar meal in an experiment that lasted 2 months.

The present results seem to confirm the good quality of the protein of rapeseed presscake meal, but its utilisation by the rat is impaired by the presence of toxic substances. Further research is necessary to remove them or reduce their level before rapeseed can be used as a food.

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TABLE IV
Available lysine, net protein utilisation (*NPU*) and true digestibility of meal

Origin	Available lysine, g/100g crude protein	*NPU*	True digestibility
Industry A:			
A–1	4·50		
A–2	4·40		
A–3	4·38		
A–4	4·41		
Mean	4·42	41	68
Industry B:			
B–1	3·90		
B–2	3·97		
B–3	4·16		
B–4	4·10		
Mean	4·03	39	66
Industry C:			
C–1	4·60		
C–2	4·58		
C–3	4·51		
C–4	4·61		
Mean	4·58	39	65

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RAPESEED MEAL

III.*—A simple method for detoxification

By DIGNA BALLESTER,† R. RODRIGO,† J. NAKOUZI,† C. O. CHICHESTER, E. YÁÑEZ** and F. MÖNCKEBERG**

Two types of treatments of presscake meal of rapeseed (*Brassica napus*), steaming and water extraction, were tested to remove the thioglucosides responsible for its toxicity. The efficiency of each was judged by the residual concentration of isothiocyanates and oxazolidinethiones and the biological quality, available lysine, net protein utilisation and digestibility in rats.

Double water extraction gave the best results. It resulted in a reduction of 84% in oxazolidinethiones, the factor directly responsible for toxicity, and 77% in isothiocyanates. Net protein utilisation increased to levels corresponding to those of animal proteins, from 40 to 69%. The improvement in digestibility was reflected in the growth of rats.

Steaming and simple water extraction, however, reduced toxicity only slightly and improved biological quality very little.

The greatest change of the gross chemical composition in treated samples was observed in crude fibre, which appeared to increase.

Introduction

In a previous paper[1] it was reported that rapeseed presscake meal has a high protein content with a well balanced amino acid pattern, but that its biological quality is impaired by the toxicity due to the presence of isothiocyanates [3-butenyl isothiocyanate] and oxazolidinethiones [L-5-vinyl-2-oxazolidinethione] (VTO), which are released from the thioglucocides upon enzymic hydrolysis.

A number of methods have been proposed to remove the toxicity from the meal, but the majority of them have not been completely successful.[2-4] Since rapeseed is widely cultivated as an oilseed, the large quantities of protein left after oil extraction make it worth considerable effort to render it useful as a protein source for animal and/or human feeding.

This paper is concerned with attempts, based on steaming and water extraction, to remove the water-soluble thioglucosides from the meal.

Experimental

Materials

A pool made from 12 samples of rapeseed meal from 3 edible-oil factories was used to test two types of treatments, steaming and water extraction, as described in Table I. In steaming, 1 part of meal and 2 parts of water were used, while in water extraction the ratio was 1:5. After each treatment, the samples were filtered and dried at 50°, ground and stored in a cold room until chemical and biological analyses were performed.

Methods

Chemical analysis

Water, total ash, calcium, phosphorus, iron, ether extract, crude fibre, nitrogen, available lysine, isothiocyanates, and oxazolidinethiones were determined by methods reported elsewhere.[1]

Biological tests

Net protein utilisation (*NPU*) of the protein and digestibility were measured in all samples according to established methods.[5]

Results

The gross chemical composition of the samples was not greatly influenced by different treatments (Table II). The protein content remained unchanged in all treated samples, as did ether extract and total ash, even when slight increases in calcium and iron were observed. The significant increase in crude fibre can be ascribed to partial dissolution of carbohydrates. However, the level at which rapeseed meal would be included in rations would not cause undesirable effects.

Table III shows the levels of isothiocyanate, VTO, available lysine, *NPU* and digestibility. In all cases a decrease in the isothiocyanate and VTO contents was observed. Similarly, the available lysine values showed a slight decrease. However, *NPU* improved markedly, the highest values being those obtained by double extraction in water. This effect was paralleled by an increase in digestibility values. The efficiency of the different treatments was also reflected in the growth of rats.

TABLE I

Treatments used to remove the toxic factors of rapeseed presscake meal

Sample	Treatment
T-1(a)	Indirect steaming, 20 lb/in² at 60°c for 3 h
T-1(b)	Direct steaming, 20 lb/in² at 100°c for 3 h
T-2(a)	Water extraction at room temperature for 10 h
T-2(b)	Water extraction at room temperature for 12 h
T-2(c)	Water extraction at room temperature for 14 h
T-3(a)	Water extraction for 8 h followed by a second extraction of 2 h at room temperature
T-3(b)	Water extraction for 8 h followed by a second extraction of 2 h at 50°c
T-4	Water extraction for 12 h followed by a second extraction of 3 h at room temperature
T-5	Water extraction for 14 h followed by a second extraction of 1 h at room temperature

* Part II: Preceding paper

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TABLE II

Chemical composition and calorie content of rapeseed presscake meal before and after the different treatments
% in dry matter

Sample	Total ash, %	Protein (N × 6·25)	Ether extract, g/100 g	Crude fibre, g/100 g	Ca, mg	P, mg	Fe, mg	N-free extract	Calories, kcal/100 g
Original	6·6	37·2	2·9	14·8	698	1093	17	38·5	325
T-1(a)	6·3	38·9	2·0	18·1	759	1143	18	34·7	312
T-1(b)	6·1	39·7	2·8	19·7	838	1215	17	31·7	311
T-2(a)	6·2	35·3	2·2	19·1	843	1011	16	37·2	310
T-2(b)	6·2	35·6	2·1	18·7	892	1035	18	37·4	311
T-2(c)	6·3	35·4	2·2	19·0	847	996	16	37·1	310
T-3(a)	5·9	35·8	2·4	19·5	864	945	20	36·4	310
T-3(b)	5·7	35·0	2·3	19·2	873	901	22	37·8	312
T-4	6·0	35·6	2·2	20·9	941	1128	17	35·3	303
T-5	5·5	36·4	2·7	19·5	992	1133	17	35·9	313

TABLE III

Effect of the different treatments upon the biological quality and isothiocyanates and thio-oxazolidones (VTO) of meal

Treatment	Available lysine, g/100 g crude protein	*NPU*	Digestibility[a]	Isothiocyanates,[b] mg/g	VTO,[c] mg/g
None	4·42	40	67	1·39	7·71
T-1(a)	3·60	49	69	0·72	6·50
T-1(b)	3·65	63	72	0·32	2·74
T-2(a)	3·61	56	66	0·69	4·01
T-2(b)	3·69	62	71	0·66	3·94
T-2(c)	3·66	58	67	0·56	3·59
T-3(a)	3·72	62	71	0·38	1·44
T-3(b)	3·80	68	75	0·35	1·37
T-4	3·96	68	77	0·37	1·32
T-5	4·10	69	77	0·33	1·23

[a] Digestibility of the protein
[b] As 3-butenyl isothiocyanate
[c] As (−)-5-vinyl-2-thio-oxazolidone

Discussion

The different treatments used in the present work lessened to varied degrees the toxicity of rapseed presscake meal, measured through the contents of isothiocyanates, VTO and biological quality in experimental animals. Indirect steaming reduced isothiocyanate content by almost 50%, but only slightly decreased the VTO level. This result is in agreement with the volatility of isothiocyanates. Since VTO is directly responsible for toxicity, the biological value was only slightly modified.

In contrast, steaming considerably reduced the levels of both isothiocyanates and VTO, and the biological value increased accordingly.

Simple water extraction reduced both isothiocyanates and VTO by almost 50% and biological quality (measured as *NPU*), rose to a value of 62%; however, this treatment was less effective than indirect steaming.

Double water extraction gave the best results, since it resulted in a reduction of 84% in VTO and 77% in isothiocyanates. Similarly, *NPU* increased to levels corresponding to those of animal proteins. The different treatments also produced a slight increase in digestibility which may have favourably influenced *NPU*.

Available lysine showed a minor reduction, especially by steam processing. One explanation of this is that exposure to heating may have led to the binding of the ε-amino group of this amino acid.

It is possible that the higher growth rate of rats fed double water-extracted meal was partly due to increased palatability of this sample, since dietary intake in this group was 3 times that of the group fed untreated material, or 0·14 and 0·04 g/rat/day respectively.

The results of the present experiments emphasise the possibility of significantly reducing the content of toxic substances in rapeseed meal. It is reasonable to expect that when the ideal conditions of water extraction have been found, complete removal will be attained.

The slight residual concentration of isothiocyanates and VTO remaining after double extraction in water did not adversely influence protein quality, inasmuch as the values found for *NPU* confirmed the high quality of the protein anticipated from amino acid analysis data.

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Fish-Protein Concentrate and Sunflower Presscake Meal as Protein Sources for Human Consumption[1]

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IN CHILE, as in most developing countries, one of the most serious medicosocial problems is chronic infant malnutrition, retarding all development and growth (1) and promoting low psychological performance (2–4) and high susceptibility to infection (5, 6). At 1 year old, about 40% of Chilean children are under the 10th Iowa percentile (7) in either weight or height. This figure rises to 70% at age 7 (7), remaining there throughout school age and adolescence.

In Chilean families of low economic status, children in the 1st year of life receive the same food as the rest of the family. The protein and calories may be sufficient for adults but not for the growing child. A high quality protein must be added to the diet, especially in the more vulnerable growth period.

The current national program for free distribution of powdered milk for infants and preschool children is handicapped by insufficient milk production with little hope of rapid expansion in the near future (8). A possible remedy would be a new protein source of good biological value. At least two new unconventional protein sources appear to be available for human consumption: fish-protein concentrate (FPC) and sunflower presscake meal (SPM).

These protein sources were, therefore, studied to determine chemical composition, measure nutritional quality in both rats and infants, establish toxicity in the rat, and determine the short-term and long-term acceptability by preschool children of FPC, SPM, FPC-enriched foods, and various mixtures containing FPC and SPM.

MATERIALS AND METHODS

The FPC was a defatted deodorized fish flour manufactured in Quintero, Chile, from a local hake called "merluza" (*Merluccius gayi*) (9).

The SPM was a by-product of the edible-oil industry, which uses a combination of expeller and solvent extraction of the seeds of *Helianthus annus* with a mixture of hexane and heptane.

The dried skim milk (DSM) was a commercial sample containing 33.6% protein.

The wheat flour was made from *Triticum durum* known locally as "harina candeal."

The bread was ordinary bread prepared in an experimental bakery according to the following formula (in grams): wheat flour, 100; yeast powder, 2; sodium chloride, 2; and sugar, 1.

The bread enriched with FPC contained 3, 6, 9, and 12% FPC, prepared by substituting

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FPC for wheat flour. All samples were cut in slices and then ground for chemical analysis.

The roasted whole-wheat flour was a fine flour produced by grinding from the roasted grain.

The roasted whole-wheat flour enriched with FPC contained 10% FPC. This mixture can be made into a gruel by adding cold water. The uncooked gruel is eaten with sugar.

Spaghetti was prepared in a factory according to the following formula: semolina, 100 kg and water, 34 liters.

Spaghetti enriched with FPC was prepared by substituting FPC for semolina at the 10% level in the formula of the ordinary spaghetti. The usual method involves preparation of a dough, lamination, cutting, and drying at 37 C for 18 hr. Because of a higher absorbance of water, the FPC-enriched dough had to be dried for 36 hr.

The three protein-rich mixtures tested were formulated (in grams) as follows:

1) MM 18: FPC, 15; SPM, 30; and wheat flour, 55

2) MM 23: FPC, 15; SPM, 30; dried skim milk, 15; and wheat flour, 40

3) MM 25: SPM, 50; dried skim milk, 25; and roasted whole-wheat flour, 25.

a) Chemical analysis. Water, total ash, ether extract, nitrogen, and crude fiber were determined by the Association of Official Agricultural Chemists methods (10). The calorie content of all samples was calculated by using the factors 4, 9, and 4 of Atwater.

b) Studies in rats. The biological quality of the materials was measured as net protein utilization in rats according to the method of Miller and Bender (11)—either in diets that supplied 10% of protein calories (NPU_{10}), or as such, i.e., net protein utilization operative (NPUop). Protein values measured as net dietary protein calories percent (NDpCal%) were calculated by multiplying NPUop by percentage of protein calories (P), according to Platt et al. (12). *Chemical scores* were calculated according to FAO (13) and protein calories percentage (P) according to Platt, Miller and Payne (12).

c) Toxicity tests. To investigate possible toxicity, three different groups of weanling rats were fed the following diets for 6 months: milk-casein (control group); FPC; and SPM at 20% protein calories. Food intake and weight gains were determined weekly. At the end of the 6-month period, weights were determined for liver, heart, spleen, kidneys, and adrenal glands, and histological studies were made of liver, thyroid, hypothalamus, adrenal glands, pancreas, lungs, heart, kidney, skeletal muscle, skin, bone, bone marrow, stomach, small intestine, cartilage, lymphatics, and spleen.

d) Nutritional value of FPC in infants. An infant formula was prepared in which FPC was the only protein source. The formula contained (in grams): FPC, 15; lactose, 35; sucrose, 19; vitaminized cornstarch,[4] 17; butter, 12; and salts, 2.[5]

This preparation, suspended at 22.5% in water, supplied 90 kcal and 2.4 g protein/100 ml. Twelve normal infants 2½–5 months old were given this formula for 30–90 days, with an average daily protein intake of 3.6 g/kg per day. Nine of them were offered FPC manufactured in the Quintero plant, and the other three received VioBin FPC. Biochemical analysis was made of blood, urine, and stools by usual methods. Weight and height changes were recorded.

To determine the minimal FPC level required to produce normal growth, 4 infants, 2–6 months old, were given the same mixture for 2 months, maintaining a constant calorie intake for three periods of 20 days, starting with 3 g protein/kg per day and scaling down to 2.5 and 2.0 g/kg per day.

e) Protein-rich mixtures in infant and child feeding. Twelve infants 2½–4½ months old were fed only a mixture containing FPC and SPM (MM 18) with added vitaminized cornstarch, sunflower oil, salts, and sugar.[6] They received this formula suspended at 20% in water

[4] Vitaminized cornstarch contained: thiamine, 300 mg; riboflavin, 500 mg; pyridoxine, 200 mg; calcium pantothenate, 2,000 mg; nicotinic acid, 2,000 mg; inositol, 2,000 mg; biotin, 10 mg; folic acid, 100 mg; vitamin B_{12}, 1 mg; vitamin K, 40 mg; tocopherol, 300 mg; vitamin A, 100,000 IU; vitamin D, 25,000 IU; *p*-aminobenzoic acid, 20 mg; choline, 20 mg; and cornstarch, 1,700 g.

[5] Salt mixture contained (in grams): $CaHPO_4 \cdot H_2O$, 65; KCl, 80; NaCl, 20; $MgSO_4 \cdot 7H_2O$, 30; Fe citrate ($FeC_6H_5O_7 \cdot 3H_2O$), 4; and traces, 1. Traces (in grams): KI, 12; NaF, 10; $MnSO_4 \cdot H_2O$, 2; Cu_2Cl_2, 1; $KAl(SO_4)_2$, 1; and $ZnSO_4$, 1.

[6] This formula contained (grams): FPC, 35; SPM, 17; wheat flour, 12; vitaminized cornstarch, 18; sunflower oil, 18; salts, 2; and sugar, 24.5.

for 11 weeks. One hundred milliliters contained 89 kcal and 3.2 g protein. Food and calorie intake and weight gain were determined daily. Initial and final height was also measured. Biochemical analysis was made of blood, urine, and stools.

To determine the effects of long-term addition of protein, protein mixtures containing FPC and SPM (MM 23 and MM 25) were given for 6 months to 80 preschool children (14). Growth, tolerance, and safety were studied. The experiment was performed at a kindergarten with children of low economic condition, 2–7 years old. They received three meals daily. At 9 AM and 4 PM the children were given 40 g of mixture MM 25 in 200 ml of water with 20 g sugar added. At lunch-time 20 g of mixture MM 23 per ration was added and cooked together with the ordinary meal. It was assumed that the children received another meal at home. These mixtures, fed 5 days a week, supplied 70% of the daily protein intake.

On the 6th day the children were given the ordinary meal, without supplementation, and this was taken as "control diet."

Acceptability was measured by weighing the daily intake of each child on experimental and control diets. In the same way their dail calorie and protein intake was measured. Hem globin, hematocrit, carotenemia, plasma pr teins, albumin, globulins, and hepatic floccul tion tests were made at the beginning and at th end of the experiment. Height was measure every 20 days.

f) Acceptability of FPC-enriched foods. Brea enriched with FPC at 3, 6, 9, and 12% w tested for acceptability by 500 schoolchildre and adults by the method of paired compariso Enriched spaghetti was tested for 3 months at school where ordinary spaghetti was a freque dish (three times a week). In the same wa roasted whole-wheat flour enriched with FP was assayed in a preliminary trial in 300 ch dren 9–14 years old.

RESULTS

Chemical Analysis

The chemical composition of all m terials tested is given in Table I. The FP manufactured in Quintero (Chile) has low fat content but a high protein le (14). The SPM has a lower protein co

TABLE I

Composition of assayed materials (g/100 g)

Material	H_2O	Ash	Ether extract	Crude fiber	Protein N × 6.25	Nonnitrogenous extract	Calorie kcal/100
Basic materials							
Fish-protein concentrate (FPC)	6.9	15.5	0.2		77.5		312
Sunflower presscake meal (SPM)	6.8	8.2	1.7	10.8	42.8	29.7	305
Enriched foods							
Bread	9.5	2.2	0.4		10.1	77.8	355
Bread + 3% FPC	6.7	1.8	0.4		12.2	79.9	389
Bread + 6% FPC	6.8	2.5	0.4		13.7	76.4	
Bread + 9% FPC	5.3	2.7	0.4		15.6	76.0	
Bread + 12% FPC	5.6	3.4	0.4		17.7	72.9	
Bread + 12% dried skim milk (DSM)	5.7	3.2	0.5		11.3	79.3	
Spaghetti	11.5	4.0	0.6		10.2	73.7	339
Spaghetti + 10% FPC	10.6	6.4	0.8		16.1	66.1	336
Roasted whole-wheat meal	4.6	1.3	2.1		8.5	83.5	387
Roasted whole-wheat meal + 10% FPC	3.8	2.7	1.8		11.1	80.6	383
Protein-rich mixtures							
MM 18	9.4	8.0	3.9	2.7	31.8	44.3	340
MM 23	8.2	8.7	2.4	2.6	34.9	43.2	384
MM 25	6.5	6.9	3.5	4.3	30.4	48.4	347

tent, and its high content of crude fiber could make it objectionable for human feeding, especially for the young.

As expected, protein concentration was increased in FPC-enriched foods such as bread, spaghetti, and roasted whole-wheat meal. The protein-rich mixtures studied had protein concentrations of 30.4–34.9%. Mixtures MM 18 and MM 23 had a similar crude-fiber content, whereas MM 25 had a higher fiber content because it contained more SPM. The calorie contents of the foods, enriched foods, and mixtures were quite similar and ranged from 336 to 389 kcal/100 g (Table I).

Studies in Rats

Table II shows the results of the biological experiments with rats. The biological quality of FPC is high, and of the same order of casein (12). Dried skim milk showed a high value of NPU_{10}. The SPM is of rather lower quality, but supplementing it with FPC increases its NPU_{10} to a value only slightly inferior to that of FPC.

Ordinary wheat flour and durum flour were similar in NPUop, but durum had twice the NDpCal% value of the other flour. Enriched foods in general showed a marked increase in NPUop, NDpCal%, and chemical score. The protein-rich mixtures had low values of NPUop but, when tested at the 10% level of protein calories, their biological utilization rises to levels comparable with those of animal proteins, FPC, and dried skim milk. Chemical scores for these mixtures closely parallel NPU values. The NDpCal% values corroborate the high biological quality of these mixtures.

Toxicity Tests

Toxicity tests revealed that rat growth rates were similar, but slightly lower with FPC and SPM than with a mixture of casein-milk at the same level of protein calories (Fig. 1). Some organs differ significantly in weight in rats fed FPC or SPM, or both (Table III). We cannot explain these differences, especially since histological studies revealed no alteration of any kind in any of the groups.

Table II

Biological quality of protein sources, enriched foods, and protein-rich mixtures

	P	NPUop	NDpCal %	NPU_{10}	Score
Basic materials					
FPC	38.0	9	8.8	70	71
SPM	34.7	22	7.6	51	64
SPM + FPC				64	70
DSM				80	78
Wheat flour	8.4	39	3.3		52
Durum wheat flour	16.7	40	6.7		59
Enriched foods					
Bread	10.4	35	3.6		57
Bread + 6% FPC	14.3	43	6.2		75
Bread + 12% FPC	16.9	48	8.1		73
Bread + 12% DSM	13.5	45	6.1		
Spaghetti	11.9	38	4.5		47
Spaghetti + 10% FPC	19.1	47	9.0		73
Roasted whole-wheat meal	8.8	41	3.6		47
Roasted whole-wheat meal + 10% FPC	11.6	71	8.2		72
Mixtures					
MM 18	35.0	35	12.3	76	81
MM 23	40.0	32	12.8	75	81
MM 25	35.0	30	10.5	66	74

P = protein calories as percent total calories.
NPUop = net protein-utilization operative.
NDpCal % = net dietary protein calories %.
NPU_{10} = net protein utilization at 10% protein calories.
Score = chemical score of basic materials, FPC-enriched foods, and protein-rich mixtures.

Biological Value of FPC in Infants

Figure 2 and Table IV show weight and height increases of infants receiving FPC as the sole source of protein. Table V shows hematological tests of the same infants.

Table VI shows that even 2.5 g of FPC/kg per day can support normal growth (corresponding to 7.4% protein calories), whereas growth was drastically reduced when this level was lowered to 2.0 g/kg per day.

Protein-Rich Mixtures in Infant and Child Feeding

Figure 3 and Table VII show weight and height changes of 12 infants fed a mixture of FPC and SPM for 11 weeks. Table VIII shows biochemical tests for the same group.

In the study involving kindergarten children (ages 2–7) who received a protein supplement of FPC or SPM, or both, for 6 months, statistically significant increases in height above that considered normal were observed. Table IX shows hematological findings for this group.

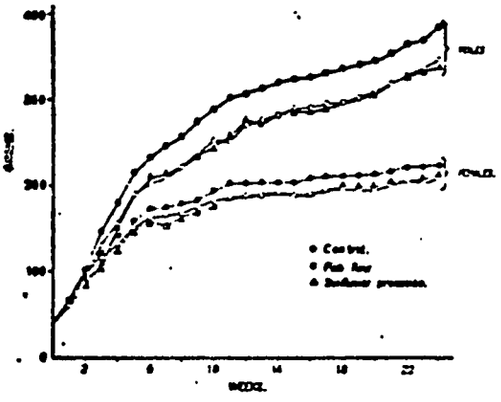

FIG. 1. Growth of rats, fed for 6 months diets based on milk-casein (control group), FPC, and SPM at 20% protein calories.

Acceptability of FPC-Enriched Foods

In tests of acceptance by adults and children, bread enriched with 3% FPC did not differ significantly from the usual product; at 6% enrichment, however, clear differences could be detected in the more critical people influenced by the darker color. Thus, when color had no importance bread enriched with 9% FPC had excellent acceptance by schoolchildren. At 12% enrichment the taste was "different but good," and was still accepted by the adults.

Enriched spaghetti was tested for acceptability by 150 adults of a hospital staff and 150 patients. The "acceptance or rejection" method indicated complete acceptance by the staff; the patients, who showed some rejection of ordinary spaghetti, showed no greater rejection of FPC-enriched spaghetti. In a longer duration (3 month) acceptability test at school where spaghetti was a principal dish (times a week), leftovers of enriched spaghetti were not noticeably greater than usual.

TABLE III

Weights of organs (g/100 g) of rats fed casein-powdered milk (control), FPC, and SPM 20% protein calories for 6 months

	Liver		Spleen		Adrenal glands		Kidneys		Heart	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Fema
Casein-milk (14 males, 14 females)	2.92 ± 0.13*	2.65 ± 0.08	0.13 ± 0.04	0.17 ± 0.00	0.014 ± 0.00	0.026 ± 0.00	0.59 ± 0.00	0.63 ± 0.01	0.32 ± 0.01	0.32 ± 0.
FPC (20 males, 15 females)	2.42 ± 0.04 $P<0.001$	2.57 ± 0.05	0.13 ± 0.00	0.15 ± 0.01	0.016 ± 0.00 $P<0.05$	0.024 ± 0.00	0.63 ± 0.02 $P<0.01$	0.69 ± 0.01 $P<0.01$	0.29 ± 0.02	0.32 ± 0
SPM (17 males, 16 females)	2.88 ± 0.05	2.91 ± 0.09 $P<0.05$	0.15 ± 0.01 $P<0.02$	0.19 ± 0.01	0.017 ± 0.00 $P<0.02$	0.028 ± 0.00	0.64 ± 0.02 $P<0.05$	0.72 ± 0.03 $P<0.02$	0.28 ± 0.02	0.32 ± 0

* Mean ± standard error of the mean.

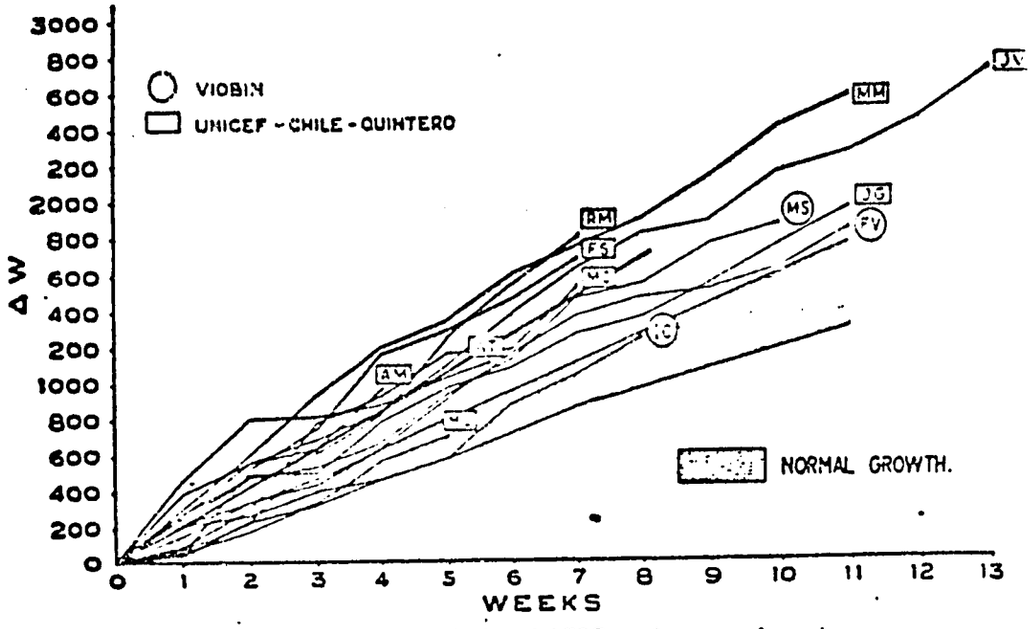

Fig. 2. Weight increment of infants fed FPC as sole source of protein.

TABLE IV

Weight and height changes in 12 infants fed FPC as the only source of dietary protein

Child	Age at start, months	Days of feeding	Weight increase, g/day	Height increase, cm	Protein intake, g/kg per day	Calorie intake, cal/kg per day
Quintero FPC						
FS	3.0	50	33	3.5	3.9	148
MM	3.5	76	35	5.0	3.8	152
MC	3.0	50	27	4.0	4.2	160
JG	4.5	77	35	5.0	3.8	146
AM	3.5	30	34	3.0	3.4	135
RM	3.5	50	36	4.0	4.0	153
MM	6.5	36	20	3.0	3.2	121
AT	2.5	40	30	2.5	3.7	152
JV	3.0	90	31	6.0	3.5	147
VioBin FPC						
MS	4.0	75	23	5.0	3.3	147
FV	5.0	77	22	5.5	3.2	144
IC	4.0	60	20	6.0	3.8	170

TABLE V

Initial and final hematological values in 1 normal infants fed FPC as the only source of protein for 3 months

	Initial	Final
Hematocrit, %	33.5 ± 0.1*	34.6 ± 1
Hemoglobin, g/100 ml	10.0 ± 0.0	10.4 ± 0.
Plasma proteins, g/100 ml	6.2 ± 0.1	6.2 ± 0
Albumin, g/100 ml	4.3 ± 0.1	4.1 ± 0
Albumin-globulin	2.3 ± 0.1	2.1 ± 0
Bilirubin, mg/100 ml	0.5	0.4
Thymol turbidity units	4.6 ± 0.1	5.8 ± 0
Calcium, mg/100 ml	9.9 ± 0.1	10.3 ± 0
Phosphorus, mg/100 ml	6.1 ± 0.3	6.1 ± 0
Alkaline phosphatase, King and Armstrong units	47.4 ± 4.3	34.7 ± 4
Zinc sulfate turbidity units	4.9 ± 0.1	8.0 ± 0

* Mean ± standard error of the mean.

Enriched and unenriched roasted whole-wheat flour did not differ in acceptability by 300 children, 9–14 years old.

Mixtures given to infants had excellent acceptability and tolerance, similar to cow's milk. Stools were of normal consistency when infants were fed either FPC, as t sole source of protein, or sunflower m ture. The stools were dark green (thou with no repulsive smell). The SPM p duced more compact stools than milk FPC alone.

The acceptability of enriched mixtures by preschool children was very good as measured by the weight of daily intake on experimental and control diets (Fig. 4). There were no gastrointestinal disturbances, and stools were of normal appearance.

DISCUSSION

Unconventional proteins cannot by themselves solve the problem of malnutrition but can be useful while steps are taken toward permanent corrections. New protein sources that meet the criteria of low-cost foods for children are FPC and SPM. The FPC appears to be an important protein source because of abundant raw material,

TABLE VI

Effect of different levels of FPC protein on the weight increase of 4 infants, ages 2–6 months, over a 60-day period

Protein intake, g/kg per day	Calorie intake, kcal/kg per day	Protein calories, %	Weight increase, g/day[a]
3.5[b]	145	10.6	28
3.0	130	9.3	30
2.5	130	7.4	26
2.0	130	6.2	13

[a] Calculated as an average over 15 days.
[b] Values obtained in earlier experiments on 12 infants, ages 2.5–5 months.

TABLE VII

Weight and height changes in 12 infants fed FPC and SPM (MM 18) as the only source of protein

Child	Age at start, months	Days on diet	Weight increase, g/day	Height increase, cm	Protein intake, g/kg per day	Calorie intake, kcal/kg per day
DN	3.5	88	26	11	5.6	154
EP	3.0	88	28	5	5.5	153
RS	3.5	88	27	7	5.6	156
GD	2.5	74	29	7	5.4	150
MCH	4.5	73	32	6	5.5	160
SA	2.0	84	23	8	5.5	155
GP	3.3	89	19	7	5.3	147
MH	3.3	48	18	4	4.9	139
GV	2.0	80	20	7	5.8	167
AN	2.5	48	21	5	5.6	157
AS	2.0	54	21	3	5.9	167
MA	4.5	78	15	5	5.1	145

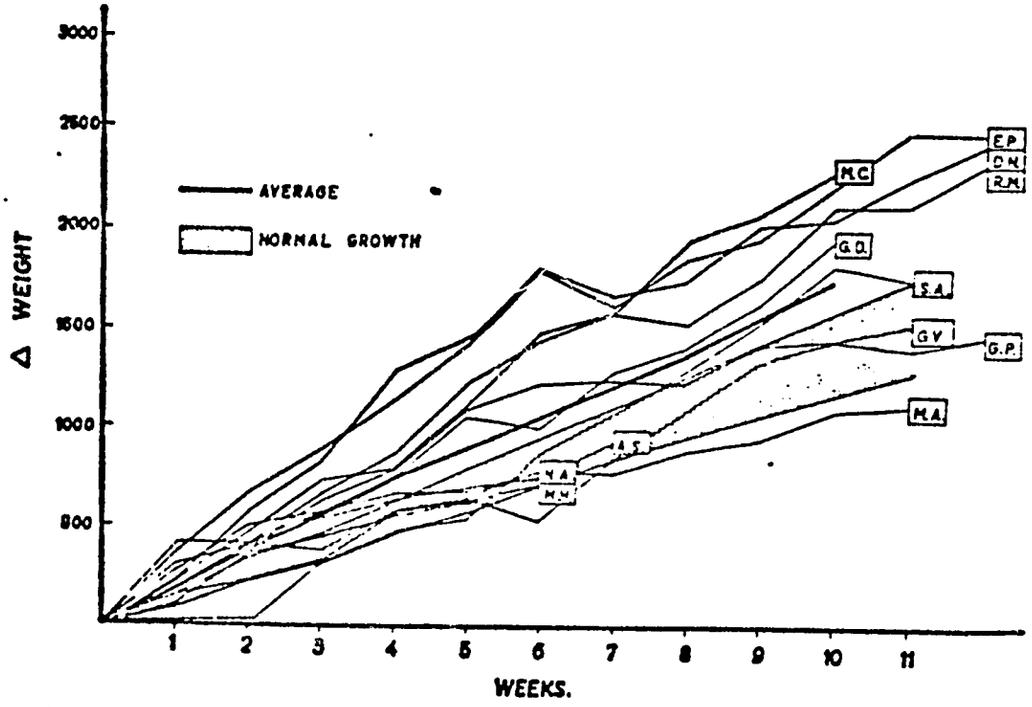

FIG. 3. Weight increment in 12 infants fed formula MM 18 (FPC and SPM) as sole source of protein.

high protein content, good biological value, and absence of toxicity. Its high, available lysine makes it valuable for improving the biological value of poorer proteins, especially those of cereals.

These results would suggest the feasibility of enriching some common foods and significantly increasing their biological quality measured as net protein utilization, chemical score, and NDpCal%. This is direct evidence of a higher concentration and better amino acid pattern of enriched foodstuffs.

The good quality of this flour was evident in the growth rate of infants. Daily weight increases averaged 28 g/day, in contrast to 20 g/day on cow's milk. Height increases were 2.2 cm/month, also higher than normal.

The SPM contains about 45% protein, and its first-limiting amino acid is lysine. As could have been expected, its biological quality was improved through supplementation with FPC. The blending of FPC and SPM (MM 18) produced normal growth when it was the only source of dietary protein of infants.

TABLE VIII

Initial and final hematological values in 12 infants fed a mixture of FPC and SPM (MM 18) as the only source of protein

	Initial	Final
Hematocrit, %	31.0 ± 1.0	33.0 ± 0.7
Hemoglobin, g/100 ml	9.6 ± 0.3	10.1 ± 0.3
Albumin, g/100 ml	3.9 ± 0.2	4.4 ± 0.1
Albumin–globulin	2.4 ± 0.1	2.2 ± 0.1
Plasma proteins, g/100 ml	5.6 ± 0.1	6.4 ± 0.2
Bilirubin, mg/100 ml	0.32 ± 0.03	0.31 ± 0.03
Thymol turbidity units	2.5 ± 0.5	4.1 ± 0.4
Zinc sulfate turbidity units	3.1 ± 0.5	5.5 ± 0.3
Calcium, mg/100 ml	9.7 ± 0.2	9.6 ± 0.2
Phosphorus, mg/100 ml	6.1 ± 0.1	5.9 ± 0.2
Alkaline phosphatase, King and Armstrong units	43.6 ± 4.4	46.6 ± 4.6

Values are means ± standard error.

TABLE IX

Initial and final hematological values in 80 children (ages 2–7) fed mixtures MM 23 and MM 25 for 6 months

	Initial	Final
Hematocrit, %	38.2 ± 2.0	37.8 ± 1.9
Hemoglobin, g/100 ml	12.1 ± 2.6	11.3 ± 1.6
Serum carotene	143.0 ± 37.6	174.0 ± 65.7
Plasma proteins, g/100 ml	6.3 ± 0.7	6.5 ± 0.6
Albumin, g/100 ml	3.8 ± 0.6	4.0 ± 0.5
Albumin–globulin	1.7 ± 0.7	1.7 ± 0.7
Thymol turbidity units	1.0 ± 0.8	1.7 ± 1.0

Values are means ± standard deviation.

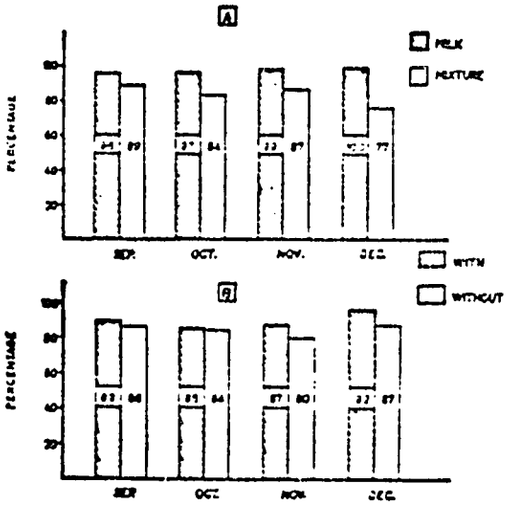

FIG. 4. *A:* Percentage of acceptability of mixtures MM 25 and *B:* MM 23 by 80 preschool children during 4 months.

When 70% of the daily dietary protein intake of children was replaced by mixtures MM 23 and MM 25, growth was maintained within the corresponding percentile although some reached the next superior percentile.

SUMMARY

Chemical composition, biological quality of the protein, and toxicity were determined for FPC, SPM, FPC-enriched food,

and mixtures based on these concentrates. Acceptability and tolerance were tested in human trials. The FPC improved the biological quality of cereal-based foods. When fed as the only source of protein, FPC produced normal growth in infants. Mixtures containing FPC and SPM showed a high net protein utilization in the rat and produced normal growth in children when they supplied 70% of the total daily dietary protein.

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APPENDIX 2

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APPENDIX 3

PRELIMINARY REPORT ON A NEW PROTEIN-RICH MIXTURE CALLED "FORTESAN"

A new infant food called Fortesan has been worked on in the Laboratorio de Investigaciones Pediátricas de la Facultad de Medicina de la Universidad de Chile in Santiago. A basic ingredient of Fortesan is WSB donated by C.A.R.E.

Previous to the formulation of this mixture the Laboratorio de Investigaciones Pediátricas conducted a study of the WSB mixture which consisted of a chemical analysis, biological quality studies in both rats and infants and a 3-month acceptability test in a nursery school. The results were acceptable considering WSB is a vegetable mixture.

1.- The NPU value was 62 compared with 70 por casein

2.- The growth of infants who received WSB as the only protein source, was inadecuate.

3.- The acceptability test in pre-school children gave generally good results. Allergy and digestive intolerance symptoms were not observed.

From these results it was decided that WSB could be used as the base of a new formula for children with the following modifications:

1) The addition of 25% dried skim milk to balance the aminoacid score.

2) Submit it to an extrusion process to make it more soluble ans stable in water. This treatment also improves the texture and taste of the product.

hydrolyzed, they can be observed more rapidly, especially in the case of small infants.

To test whether the hydrolysis of the starch was adequate the mixture was tested in five newly born infants. They were given 4 g/kg/day of protein. The caloric requirement was completed with vegetable oil. No symptoms of digestive intolerance were observed and growth rate was normal.

Based on the results obtained in these tests a new formula called Fortesan was designed. The composition of Fortesan is as follows:

Extruded WSB	70
Dried skim milk	25
Cocoa powder	5

Chemical analysis (%)

Moisture	6.0
Ash	6.6
Protein	23.6
Fat	4.0
Crude fiber	1.7
Calories	345

Vitamins (calculated) (in 100 grams.

B 1	0.57 mg
B 2	0.80 mg
Niacin	4.2 mg

B 6	0.12 mg
A	1170 I.U.
D	140 I.U.
B 12	traces
E	1.0 I.U.

Minerals (in 100 grsms) (calculated)

Ca	800 mg
P	450 mg
Fe	7.4 mg
I	38 mcg

Biological quality of the protein:

	PER	NPU
Fortesan	2.65	70
Casein	2.87	72

At present a long term acceptability Test is being conducted in 2000 pre-school children in the province of Curicó in order to test acceptability of both the child and the mother. This experiment will last 1 year. Every child receives 1.5 kg of Fortesan every month.

Fortesan has been canned in metal containers simulating those of Leche Nido made by Nestlé, following the advise of a group of marketing experts who proposed the name of Fortesan and designed the label used.

Chiprodal (Nestle Chile) conducted a shelf-life study and a test on organoleptic properties of the product according to the methodology they follow for their own

products. The results were satisfactory, except for a slight "old-age" odor which was present in WSB before processing.

Excerpted from the Project Semi-Annual Report (September 1970)

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)

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

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.