UTILISATION OF NON-EDIBLE SEEDS AND SEED-CAKES—I.

VEGETABLE CASEIN FROM *PONGAMIA GLABRA* AND ITS APPLICATIONS.

By N. Srinivasan and V. Subrahmanyan.

During recent years, the rapid advance in fat technology has created a widening market for vegetable oils and gained an added importance to the oil-crushing industry. The seed-cake is the most valuable by-product of this industry and its utilisation has been, therefore, the subject of a number of enquiries in different parts of the world, particularly Europe and America.

Although the use of seed-cakes as animal feeds or organic manures is well known, they do not yet command good prices in many parts of India. Even edible cakes often find only limited demand and have to be stored for a considerable length of time as the result of which they become subject to insect and fungus attack and are rendered unfit for animal consumption. If fed to animals, such cakes lead to serious disorders such as inflammation of the digestive organs. The quality of milk and butter are also spoiled. Many of them contain bitter or poisonous ingredients and fetch low prices as organic manures. There are also others, which either possess poor manurial value or are otherwise unsatisfactory, and as such, can hardly have any demand since no use has so far been found for them. In this manner, the producer is generally left with large stocks of cakes which cannot be easily disposed of, so that some other use must be found for them, particularly in India, if the oil-crushing industry is to prove flourishing.

In addition to seed-cakes, there are also large quantities of various kinds of seeds, which are available in tropical forests and for which no satisfactory use, has so far been found. They also contain certain valuable constituents and can be used in the same manner as seed-cakes.

The application of seeds and seed-cakes in different industries does not however find sufficient mention in literature, partly on account of the fact that many of the researches have not been published (being maintained as trade secrets) and partly because the patent specifications relating to the subject are too sketchy for any detailed discussion of the principles and methods involved.

The commoner seed-cakes contain carbohydrates (chiefly starch and cellulose), proteins and other forms of nitrogen, small quantities of unexpressed oil, minerals and in some cases, certain other constituents which render the cakes non-edible. In most cases the protein and starch would appear to be the most valuable ingredients. Many of the seeds and seed-cakes contain over 4 per cent. of nitrogen which corresponds to about 25 per cent. of protein. It is well known that many proteins possess properties similar to those of milk casein, which finds extensive application, in Western countries, in various industries such as paper manufacture, paints and plastics, in the preparation of adhesives, soap, leather, insecticides and a number of other articles of practical utility. Since in many parts of the tropics and particularly in India, the supply of milk is not sufficient even to meet the existing demand as an article of food, it was considered desirable to extract the proteins (vegetable casein) from the different seeds and seed-cakes and to standardise the conditions for their utilisation in the various industries such as those mentioned above. The present communication relates to the seed-cake of *Pongamia glabra* (Vernacular names: Sanskrit, *Karanja* or *Naktamla*; Hindi, *Sukchain*; Bengali, *Dahar Karanja*; Marathi, *Karanja*; Tamil, *Pungam*; Canarese, *Honge*) which is available in fairly large quantities in most parts of India. Some space is also devoted to discussion of the application of vegetable casein in the preparation of paints and plastics and as spreader for insecticidal sprays.

EXPERIMENTAL.

A number of methods have been described and a few patents taken for the preparation of proteins from seeds and seed-cakes. Unfortunately, many of them are of only academic interest, so a number of preliminary trials had to be carried out to select a few that are cheap and convenient to work with.

The first step in the preparation is the extraction of the protein; so the cake, after being sun-dried and powdered to pass the 20 mesh sieve, was treated with a number of solvents which were found from previous studies to be promising. After standing overnight, the supernatant liquids containing the protein in solution were syphoned out in each case, fresh lots of solvent added to the residue and the extraction repeated. After twice repeating the extraction, the mixture of extracts obtained from each treatment was centrifuged to separate the starch and then treated with excess of sulphuric acid or hydrochloric acid accompanied by heating. The protein which then separated was washed, centrifuged repeatedly with water to free it from dissolved impurities, and dried at 55°C in a current of air. The results are presented in Table I.

TABLE I.

Chemical used for extraction	Concentration	Acid used for precipitation	Percentage yield on the dry weight of cake	Nitrogen per cent.
Lime	Saturated solution of slaked lime	Hydrochloric	7·8	8·1
Lime	Excess of burnt lime	Hydrochloric	Dark product	
Common salt (NaCl)	1 per cent.	Sulphuric	9·8	8·9
(*a*) Common salt and (*b*) Lime	(*a*) 1 per cent. and (*b*) sat. soln.	Hydrochloric	No advantage over NaCl alone	
Caustic soda (NaOH)	0·5 per cent.	Sulphuric	24	7·8
Caustic soda (NaOH)	0·2 per cent.	Sulphuric	15·4	8·5
(*a*) Lime and (*b*) Caustic soda	(*a*) Sat. soln. and (*b*) 0·2 per cent.	Hydrochloric	No advantage over NaOH alone	
Sodium carbonate	1 per cent.	Sulphuric	23	8·2

It may be seen from the above that the hydroxide and the carbonate of soda extract the largest amounts of protein. Sodium chloride peptises only half that quantity but the product obtained by its use is slightly purer than those obtained in other cases. Its merit appears to lie in the fact that it extracts less of foreign matter than the other solvents. Slaking burnt lime together with the cake powder, did not lead to satisfactory results, the protein preparation obtained by that method being dark and impure. Nor was any advantage gained by mixing caustic soda or sodium chloride with slaked lime, so that no saving would result from the use of such mixtures. Caustic soda is nearly twice as efficient as sodium carbonate, but by a repetition of its use for extraction, dark liquors and unclean precipitates were obtained. That alkali would appear to extract, especially after the cake has soaked for some time, a number of foreign substances, so that it will not be so suitable as the yield would appear to indicate. In view of the above and the comparative cheapness of sodium carbonate, it was decided to use that chemical for subsequent extractions.

A further advantage in the use of sodium carbonate is that it can be obtained cheaply in many parts of India in the form of earth salts. Some of the provinces contain fairly large areas of alkaline soils, which, during certain seasons, particularly Spring, form white encrustations containing 10–15 per cent. of the carbonate together with small quantities of the chloride and sulphate of soda. Because of these inorganic impurities and the difficulties in concentration, the efflorescence from such soils is not suited for the manufacture of sodium carbonate. On the other hand, a solution containing a mixture of those salts would be eminently suited for the extraction of protein. The chlorides and sulphates are also protein solvents and would thus form useful supplements to the carbonate. Some experiments carried out with extracts prepared out of alkali soils available near Mandya in Mysore State showed that they were quite as satisfactory as solutions of pure carbonate.

Experiments were next carried out to determine (*a*) the effective concentration and minimum quantity of alkali required for the extraction, and (*b*) the conditions relating to the separation and filtration of the extract, particularly when large quantities of cake have to be treated.

Specimens of powdered cake prepared in the manner described above were extracted repeatedly with alkali solutions of different concentrations. As the result of these trials, it was found that extraction with 1 per cent. solution in the first instance followed by that with 0·5 per cent. solution for successive treatments was quite adequate for the purpose.

With a view to determining the minimum amount of alkali required for the extraction of the protein, some experiments were carried out repeating the treatment with dilute alkali in the manner already indicated. The extracted protein was coagulated by treatment with sulphuric acid followed by heating. The results are presented in Table II.

TABLE II.

Weight of carbonate used for extracting 100 parts of cake	Percentage yield of crude protein on dry weight of cake	Nitrogen per cent. in the preparation
10·0	38·5	5·21
2·0	38·0	5·30
1·5	39·0	5·38
1·0	36·0	5·36

It may be seen from the above that the yields, as also the nitrogen contents of the different preparations, were nearly the same in all the cases. The product obtained by repeated extraction with 10 parts of alkali was found, however, to be dark and hence unsatisfactory for a variety of purposes. The best results were obtained by the use of 1·5 parts of alkali.

After a number of trials, the following was found to be the most efficient method of extraction:—The alkali carbonate corresponding to 1 per cent. of the weight of the cake is dissolved in minimum quantity of water and the cake powder stirred into it to obtain a thick, homogeneous paste. After standing for some time, more water is added until the milky extract begins to separate from the cake. The liquid suspension containing the protein is then separated either by passing through a close-meshed sieve or, preferably, by rotating in a hydro-extractor.

Further observations also showed that prolonged soaking of the cake was unnecessary: in fact such a procedure tended to extract considerable amount of foreign matter. The yields obtained after soaking for different periods of time showed that two hours was quite sufficient for the purpose (Table III).

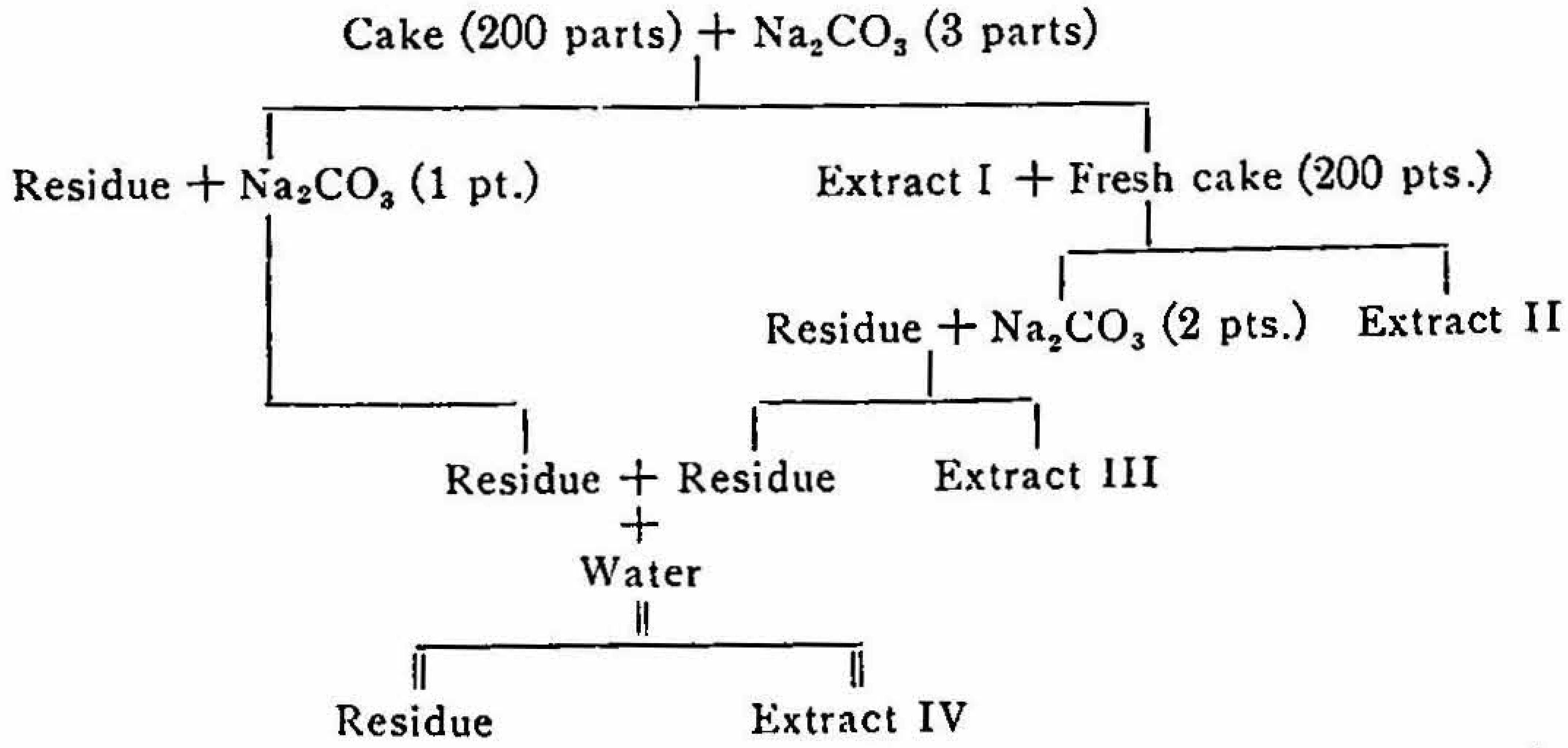
TABLE III.

Liquid soaked in	Time of soaking	Subsequent treatment	Percentage yield on the dry weight of cake	Nitrogen per cent.
Alkali soln.	Overnight	Extracted	38	5·1
Water	,,	Treated with alkali followed by extraction	39	5·2
Alkali soln.	2 Hrs.	Extracted	39	5·4

Some experiments were also carried out powdering the cake to different states of division. It was observed that coarse particles were more satisfactory to work with than fine ones. In the latter case, not only was the filtration more difficult but the protein obtained from the extract was also strongly coloured thereby showing that much undesirable foreign matter was being extracted. Warming the alkaline suspension to hasten the peptisation of the protein was not helpful either, only increasing quantities of colouring matter thus passing into solution. During the heating, part of the starch also got gelatinised so that the subsequent extraction and filtration were rendered difficult.

As the result of a number of experiments the following scheme of extraction (Table IV) was found to be the most satisfactory in practice.

TABLE IV.



Extracts I, II, III and IV were mixed for the precipitation of protein.

In the foregoing scheme, the minimum possible amount of alkali is sought to be utilised for extracting the protein. The concentration of the extract is also maintained as high as possible so that the amount of extract to be dealt with may be small. The disposal of the liquid left after the precipitation of the protein is also thus rendered less difficult.

The efficacy of the above scheme of extraction would be seen from the results of the following experiment:—The cake (500 g.) was soaked overnight in 1500 c.c. of 1 per cent. solution of sodium carbonate. The extract was then separated and the residue re-extracted a number of times with 0·5 per cent. alkali solution. After the fifth extraction, it was found that the coagulable protein in the residue was small, so the extraction was stopped. The mixed extracts were then centrifuged to remove starch, acidified and the coagulation of the protein completed by heating. The results obtained by this method of thorough extraction were compared with those obtained according to the scheme outlined in Table IV.

TABLE V.

Extract Number	Dry weight of protein preparation in grams	Nitrogen per cent. in the preparation	Percentage protein (calculated)
I	93·0	8·0	46·5
II	25·0	6·5	10·0
III, IV & V (mixed)	8·5	5·5	3·0
Total ..	126·5	..	59·5
Results obtained according to Table IV :—			
Combined extract	115	8·2	59·0

TABLE VI.

Composition of the residue left after extraction of protein.

Treatment	Weight of residue in grams	Nitrogen per cent.
Repeated extraction ..	120·0	0·52
According to Table IV ..	143·0	2·1

It may be seen from the above that in spite of the large amount of carbonate (7·0 per cent. on the weight of the cake) used, the method of exhaustive extraction after overnight soaking is less advantageous than the scheme previously outlined. Firstly, although the total amount of protein in the preparation is about the same in both the cases, the one obtained by exhaustive extraction is less pure than that prepared according to the new scheme. Secondly, the residue left after the former treatment is not only less in weight but also contains a lower percentage of nitrogen, thereby showing that a considerable part of the nitrogen passes as non-coagulable matter in the acidulated extract. In view of the above and the convenience in handling the different products in rapid succession, the scheme of treatment outlined in Table IV was followed in all subsequent extractions.

Separation of starch.—The milky extract obtained from the cake contains a fairly large amount of starch which would correspond to over 10 per cent. on the weight of the original cake. Although starch is a desirable ingredient in the preparation of water paints and a few other products, it is not so useful as the protein in other directions. It would be desirable therefore to separate it from the extract before the coagulation of the protein. With this in view, experiments were conducted examining the precipitates separating under different conditions. It was thus found that although starch separates of its own accord (in fact it is the first to separate from the alkaline extract) after standing for about 4 hours, it would be advantageous to separate it by other mechanical means even at the outset. If the spontaneous separation of starch is allowed to proceed, some of the protein is mechanically carried down by the starch and thus lost. A certain quantity also tends to deposit on top of the starchy layer and cannot be easily separated. It was found advantageous, therefore, to separate starch by centrifuging for 15 minutes at a moderate speed of about 1500 R.P.M. The loss of coagulable nitrogen was thus reduced to a minimum as may be seen from the following results (Table VII):—

TABLE VII.

Separation of starch by centrifuging.

Weight of original cake in grams	Dry weight of starchy layer in grams	Nitrogen in the preparation of starch per cent.	Loss of coagulable protein per cent.
500·0	60·0	1·0	5·0

Coagulation of the protein.—This is usually carried out by treating the alkali extract with sufficient amount of acid to render the medium slightly acid. This procedure, though theoretically sound, was not found useful in practice. Firstly, the mineral acid is highly expensive and its use would naturally raise the cost of preparation of vegetable casein considerably. Secondly, the acid required to coagulate the protein is generally very much in excess of what may be expected from the amount of sodium carbonate used for the extraction. The medium is strongly buffered and to attain the optimum hydrogen-ion concentration for the coagulation of the protein, considerable amounts of acid are required. Furthermore, it has been shown in the case of milk casein (Van Slyke and Harts, *Amer. Chem. Jour.*, 1905, **33**, 461; Van Slyke and Van Slyke, *Ibid.*, 1907, **38**, 383; Van Slyke and Van Slyke, *Jour. Biol. Chem.*, 1908, **4**, 259; Linderstrom-Lang and Kodama, *Compt. rend. Lab. Carlsberg*, 1925-27, **16**, 1) that it can absorb and even combine with large quantities of mineral acid especially in presence of the corresponding alkali salts. The vegetable protein would also appear to exhibit a similar behaviour. In one experiment it was observed that to neutralise an extract containing 40 g. of sodium carbonate nearly 60 c.cs. of sulphuric acid (1: 1) were required though the maximum required for the purpose should have been only about 40 c.cs. Moreover, the coagulation was slow and the extract had to be maintained at the optimum temperature (70°C.) for some length of time before any useful separation of protein could take place. Even then, the precipitation was incomplete. Further addition of acid or prolonged heating was useless and, in fact, disadvantageous since the precipitate was observed to deflocculate, and, being finely divided, passed out through the filter. In addition to the above, it was also noted that the preparation of protein obtained by treatment with acid possessed poor adhesive properties and thus became unsuitable for a variety of purposes.

With a view to eliminating the above difficulties and, if possible, to avoid the use of mineral acids, a number of trials were carried out investigating other methods of extraction and precipitation. Some extractions were carried out with either caustic lime or a mixture of sodium hydroxide and slaked lime and followed by passing carbon dioxide to precipitate calcium carbonate together with the protein. The procedure proved satisfactory so far as the protein in the extract was concerned but the extraction itself was rather poor, so the product obtained was mostly calcium carbonate with just a small amount of protein. In view of this inherent defect, the use of lime or mixture of lime and caustic soda was not adopted.

Precipitation of protein by addition of gypsum.—Among the different non-acid reagents that could be used for the neutralisation of alkali carbonate, gypsum appeared to be promising because it is cheap and is abundantly available in many parts of the country. It reacts with the alkali forming the carbonate of calcium which is actually used in the preparation of water paints. Experiments were carried out, therefore, adding varying quantities of gypsum to the alkaline extract and studying the effect on the reaction of the medium and coagulation of the protein. It was observed that addition of a thin suspension of gypsum in water led first to the partial dissolution of that salt in the solution followed by reaction with the alkali carbonate resulting in the precipitation of equivalent amount of calcium carbonate and formation of soluble sodium sulphate. A further quantity of gypsum then passed into solution and reacted with its equivalent of sodium carbonate. In this manner, chiefly owing to the large excess of gypsum and the steady removal of the insoluble calcium carbonate from the sphere of action, the reaction proceeded forward until the H-ion concentration favourable for the coagulation of the protein was attained. The precipitation of calcium carbonate also helped mechanically to hasten the separation of the protein. The reactions that led to the partial neutralisation of the alkali and the precipitation of the protein may be represented as follows:

(1) $$\underset{\text{(solid)}}{CaSO_4} \rightleftarrows \underset{\text{(in solution)}}{CaSO_4}$$

(2) $$\underset{\text{(in solution)}}{CaSO_4 + Na_2CO_3} \longrightarrow \underset{\substack{\downarrow \\ \text{(precipitate)}}}{CaCO_3} + Na_2SO_4$$

Influence of spontaneous fermentation on the coagulation of protein.—It was observed on certain occasions that the alkali extract of protein left standing for some time turned frothy, suggesting that some fermentation was in progress. It was also noted that in the case of extracts treated with gypsum, the fermentation started even after standing overnight thereby suggesting that the partial removal of the alkali carbonate was helpful to biological activity. With the progress of the fermentation, the frothy extract began clearing accompanied by steady deposition of the protein. The reaction became first neutral and then distinctly acid. These observations suggested that the carbohydrates and other fermentable matter extracted by the carbonate solution were being acted on by the micro-organisms introduced through water, cake, atmosphere and other channels resulting in the formation of acid. In this manner not only was the neutralisation of the alkali carbonate brought about, but the coagulation of the protein also greatly facilitated. The formation of gas (mostly carbon dioxide) was also helpful to the precipitation of protein.

Although a spontaneous fermentation of the above type has its advantages, it was felt, however, that the reactions should be so controlled that (*a*) none of the protein is hydrolysed, and (*b*) the acid production is fairly rapid so that the separation of the protein may be complete within a short period. It was considered desirable, therefore, to use a starter such as a fermentable carbohydrate together with the necessary microflora such as will be provided by a suspension of clay so that the production of organic acids and carbon dioxide may proceed rapidly without appreciably affecting the precipitation of the protein. Since ordinary soil would contain large amount of dark humic matter it was thought desirable to use white clay (kaolin) in its place. Since kaolin is actually used together with casein in certain industries, it was felt that it would make a useful addition to the preparation.

It was observed that the protein separated more readily in presence of small amounts of organic acids, such as those produced by fermentation, than on addition of proportionately larger quantities of mineral acids. With a view to explaining this phenomenon, some experiments were carried out adding different mineral as well as organic acids to suspensions containing vegetable protein and determining the quantities of acids thus taken up in each case. As observed in the case of milk casein (Van Slyke and Van Slyke, *loc. cit.*), the mineral acids were taken up by the protein while organic acids remained practically unchanged thereby suggesting that the protein did not form any complex with organic acids or, having formed one, the product was readily hydrolysed by the water present. This observation would explain the superiority of the biochemical method over that of precipitation by treatment with mineral acid.

With a view to determining the efficacy of molasses as a starter, an experiment was carried out adding it to the alkali extract in quantities corresponding to 1 per cent. on the weight of the cake together with kaolin as the source of mixed microflora for initiating the fermentation. The precipitates, which were formed after standing overnight, were separated by syphoning out the supernatant liquid, centrifuging the suspension containing the protein followed by washing repeatedly with water and finally drying at 55°C. The yield of crude protein thus obtained, as also the nitrogen content, were compared with those for the products obtained by acid precipitation and addition of gypsum respectively. The results are presented in Table VIII.

TABLE VIII.

Method of precipitation	Percentage yield of crude protein on the dry weight of the cake	Nitrogen per cent. in the preparation	True yield of protein as percentage on the dry weight of the cake
Acid (H_2SO_4) ..	38·0	5·3	12·6
Gypsum ..	42·0	5·2	13·7
Biochemical ..	40·0	5·6	14·0

The true yield of protein was calculated by multiplying the yield of crude product with the percentage of nitrogen present in it and dividing the product by 16, which is the average percentage of nitrogen in pure protein. It may be seen from those estimates that the best results were secured by the biochemical method. Treatment with gypsum yielded slightly less and that with mineral acid still less. In view of the above and the comparatively high cost and large consumption of mineral acid, that method was not considered to be satisfactory for the preparation of the protein.

It is not easy to differentiate between the effect of the purely chemical action of gypsum and that of the fermentation which accompanied its addition. Since both the processes help to neutralise the alkali and to facilitate the separation of the protein, it was considered desirable to combine them. Accordingly, gypsum and kaolin were both added as precipitants for subsequent preparations.

The adhesive properties of the preparations obtained by the three methods were compared after mixing them with suitable amounts of clay, chalk and pigment and applying the paints thus obtained to different surfaces. It was observed that the product obtained by acid precipitation would not form a smooth paste that would adhere firmly to any surface. On the other hand, the products obtained by the other two methods mixed more evenly with the added materials and adhered firmly to the surfaces to which they were applied. They also dried readily forming homogeneous films which stood rubbing or washing with water.

It is difficult to explain, at this stage, the nature of the reactions that render the acid precipitated protein so different from the other preparations, in general properties and behaviour. As already indicated, it is probable that the acid, in addition to decomposing the alkali proteinate, also enters into some combination with the liberated protein forming a product with different properties. Some of the acid adsorbed by the precipitate may also become concentrated during the drying and thus affect the quality of the product. Evidence of this may be seen in the partial charring of the acid-treated product on prolonged drying though at a moderate temperature. On the other hand, it would appear that the alkali proteinate is not seriously affected by the other methods of treatment. The medium develops only a mild acidity and the evolution of carbon dioxide hastens the precipitation, so that possibly the composition of the product is less drastically affected by such mild treatments than by heating with excess of mineral acid. This aspect of the problem is of much academic as well as practical importance and further work is in progress to improve on the conditions relating to the precipitation of the protein.

Treatment of the residual cake.—The material left after the extraction of the protein represents about 30 per cent. of the dry weight of the cake and is made up of crude fibre, colouring materials, a part of the starch and the major part of the minerals present in the original cake. It also contains about 2 per cent. of nitrogen which is mostly non-coagulable. The major part of this nitrogen can be brought into solution by prolonged treatment with alkali, but the extract thus obtained also contains large amounts of undesirable colouring matter which greatly reduces the value of the final product. Although it contains small amounts of protein the extraction of the latter is attended by a number of difficulties, so it would appear to be desirable to leave it in the residue and, if possible, find other uses for the same.

If allowed to stand by itself, the residual cake undergoes fermentation, emitting unpleasant odour. It is also overgrown with fungi, which thus cause considerable loss of organic matter and nitrogen. The cake can be rapidly dried however by spreading in thin layers followed by sun drying. If left undisturbed, the dried mass tends to form hard lumps, so it would be desirable to beat out the partially dried product in such a manner as to crumple it to a coarse powder. When the quantity of residue to be dried is large, occasional trampling with feet or treading with wooden shoes will help to break up the lumps and hasten the drying.

The drying of the liquid extract left after the separation of protein is very much more difficult, however, than that of the solid residue. Since the former contains certain valuable ingredients which can be usefully combined with the latter, subsequent experiments were devoted to drying mixtures of the two products.

Concentration of the supernatant liquid.—The liquid residue contains a considerable part of the mineral constituents, as also the bulk of the non-coagulable nitrogen, present in the original cake, together with small quantities of alkali salts of organic acids (formed during fermentation) and sodium sulphate formed through addition of gypsum. The composition of the liquid would suggest that it would be very difficult to dispose of through the usual channels of drainage while the presence of the different fertilising ingredients would point to the desirability of concentrating it in some form and utilising the product as organic manure.

A number of trials were carried out, therefore, treating the liquid in different ways. Evaporation of the thick, syrupy liquid by heating was found to be slow and tedious. The cracking and sputtering of the thick, viscous concentrate rendered heating beyond a point undesirable and even unsafe. Moreover, the final product obtained after prolonged heating was a sticky mass which was highly hygroscopic and was, in consequence, very inconvenient to handle as such. Addition of finely divided clay or such other material was useful but it tended to dilute the final product. It was considered desirable, therefore, to concentrate the liquor upto the syrupy consistency after which the solid, cake-residue could be added to it and the mixed product dried, as a whole, to yield a useful organic manure.

Some experiments were carried out pouring the supernatant liquid into shallow trays and allowing spontaneous evaporation to take place. Small quantities of antiseptics such as phenol or thymol were added to the liquid product to minimise the risk of fermentation and consequent loss of dry matter as well as nitrogen in the gaseous form. In usual practice, however, the antiseptic was not generally needed because the evaporation proceeded rapidly in thin layers so that there was no appreciable loss by fermentation. After the evaporation had proceeded to a point when the residual cake can be added to it, the latter was strewn in carefully, the product worked up to a pasty condition and then allowed to dry in the manner described already. The final product, thus obtained, was clean and dry and possessed no unpleasant odour. Being finely divided, it was more suitable for application to land than the original cake itself. The following observations (Table IX) relating to the yield and composition of the resulting manure will be of interest:—

TABLE IX.

Percentages on the dry weight of the original cake		Percentage composition on the dry weight of final product			
Solid residue	Solids in the extract	Moisture	Organic matter	N	P_2O_5
29·5	17·5	10·1	82·6	4·4	0·6

It was noted that the dry matter contents of the residue and the supernatant liquid were highly variable depending chiefly on the time of soaking with the alkali carbonate and the method adopted for subsequent extraction. Prolonged treatment led invariably to the major part of the cake being peptised by the alkali and thus passing into the supernatant liquid while, with short periods of soaking, the major part of the solid matter, including a high percentage of the non-coagulable nitrogen, were retained in the solid residue.

Further studies on the distribution of nitrogen in the cake showed that although the original computation was made on the assumption that the entire quantity of nitrogen was present as protein, yet no more than 50 per cent. was actually present in that form. Since under the conditions in which the experiments were conducted, all the proteins in the extract should normally have coagulated, it may be inferred that the non-coagulable forms of nitrogen were not really proteins but, probably, intermediary or degradation products thereof. In the present investigation the highest percentage of coagulation secured was of the order of 50 per cent., so it would be necessary to use a different factor $\frac{6 \cdot 25 \times 50}{100}$ to calculate the actual amount of protein present in Hongay seed-cake from the nitrogen content. In a like manner, each seed or seed-cake may have its own factor for protein—the coagulable forms as we understand them—so that it may be useful to conduct further systematic work on this aspect of the problem. The present convention in analytical as well as biological assay is to investigate the coagulated product and then to multiply the results with a factor based on the assumption that all the nitrogen is present in that form. Such an assumption is generally incorrect. Thus, the saline extract of a leguminous seed is not entirely made up of globulins and albumins; only a small portion is present in those forms, while the rest would appear to be made up of less complex, non-coagulable forms of nitrogen. This fact is not taken into consideration however by most workers who assume that the nitrogen is present exclusively as coagulable forms and ignore the non-coagulable ones, which may, in fact, be even more important from the chemical as well as biological point of view. Further research is needed, therefore, to throw light on the nature of the non-coagulable forms of nitrogen present in different plant as well as animal products and to determine their chemical properties and biological values.

Some experiments were carried out adding the supernatant liquid to a number of organic materials such as dried leaves and town refuse and allowing the mixture to undergo fermentation to yield a homogeneous compost as the final product. Although this procedure was clean and hygienic and did not require much technical attention, the inevitable loss of dry matter and some of the nitrogen discouraged further attempts in that direction. Moreover, as the operation also took several weeks for completion, it was considered desirable not to add any bulky organic substance, but to prepare a concentrate made up exclusively of the residual solid and the supernatant liquid.

Loss of nitrogen during the preparation of protein.—With a view to determining whether all the nitrogen originally present in the cake was recovered in the form of different finished products, some experiments were carried out studying the distribution of nitrogen at different stages during the precipitation of the protein and the preparation of the manure. After extracting the seed-cake (100 g.) with the requisite amount of alkali, the dry weight of the solid residue as also its nitrogen content were determined. The fermentation of the alkali extract was then initiated by addition of a minute quantity of clay. After two days when the precipitation was complete, the protein was separated and the nitrogen contents of the wet and sun-dried products as also that of the supernatant liquid were determined. The residual cake and a portion of the supernatant liquid were then mixed with a small quantity of clay of known nitrogen content and the product dried rapidly in the sun in the course of a day. The nitrogen content of the resulting manure was then determined. The series of operations outlined above were divided into three groups, (*a*) extraction with alkali carbonate, (*b*) precipitation of the protein, and (*c*) preparation of manure, and the nitrogen balance determined at each stage (Table X).

TABLE X.

Form of nitrogen	Total quantity of nitrogen in grams	Percentage on the nitrogen in the original cake
Extraction with alkali—		
Original cake	4·53	—
Residual ,, ...	0·67	14·8
Alkali extract	3·86	85·2
Precipitation of protein—		
Wet precipitate (148·5 g.)	2·30	50·8
Dry ,, (35·0 g.)	2·30	
Supernatant liquid (500 c.c.) ..	0·83	18·3
Total Nitrogen before precipitation ..	3·86	85·2
,, ,, after ,, ..	*3·13*	*69·1*
Loss of Nitrogen	0·73	16·1

Form of nitrogen	Total quantity of nitrogen in grams	Percentage on the nitrogen in the original cake
Preparation of manure—		
Residual solid	0·67	14·8
Supernatant liquid (350 c.c.) ..	0·58	12·8
Clay (50 g.)	0·015	0·3
Dry manure (86·5 g.)	1·27	28·0
Total Nitrogen before drying ..	1·27	28·0
,, ,, after ,, ..	1·27	28·0

It may be seen from the above that there is no loss of nitrogen either during extraction with alkali or preparation of manure. Quite considerable loss has occurred however during the fermentation of the extract. There could not have been any loss of nitrogen in the coagulable forms (mostly protein) because, as observed in an earlier part of the work, the yield of protein by acid treatment (when no fermentation could have occurred) and by the fermentation method were very nearly the same. In fact, the results (Table VIII) would show that a higher yield of coagulable nitrogen was obtained by the fermentation method than by treatment with mineral acid. It would follow, therefore, that the loss of nitrogen was mostly in the form of non-coagulable matter (mostly intermediary and degradation products of protein) which should ordinarily have passed into the supernatant liquid. As the result of this loss, the nitrogen content of the resulting manure has been lowered by 36·5 per cent.

The loss of nitrogen during the preparation of the protein raises highly important issues which are of much practical significance. Further work is in progress, therefore, to determine the forms in which nitrogen is lost and the chemical and biological mechanism of the attendant transformations. Some preliminary experiments have already shown that the loss of nitrogen occurs mostly, after the first day of the fermentation; and that the loss at the end of 24 hours was only 5 per cent. while that at the end of about 72 hours was 16 per cent. Attempts are also being made to control the conditions relating to the fermentation in such a manner that the precipitation of protein is complete within 24 hours so that the loss of nitrogen may be greatly reduced.

Large-scale preparations of protein.—The cake was crushed to a coarse powder passing the 20-mesh sieve and extracted according to the scheme outlined in Table IV. The extraction was carried out by stirring up the cake in alkali solution of the desired strength and filtering the extract through large metallic sieves. The residue left after the extraction was pressed out thoroughly to remove the liquid retained by it. An alternative procedure that was found to work satisfactorily was that of rotating the alkaline suspension of the cake in a hydro-extractor when the liquid extract was rapidly driven out by the centrifugal force. The extract thus obtained was then treated with the required quantities of gypsum and kaolin, the mixture stirred with long, wooden rods and then allowed to stand.

After two days, when the separation of the protein was complete, the supernatant liquid was syphoned out and the precipitate washed a few times by decantation. The sediment containing the mixture of protein, gypsum and kaolin was dried partly in the sun or partly in a current of hot air at 55-60°. With proper sunshine, the drying was fairly quick, but when the sky was overcast and the weather unfavourable, the drying had to be carried out under shade after first heating the product in a current of hot air for a few minutes. If in spite of the heating the product became smelly indicating further fermentation, it was found useful to treat the drying mass with some antiseptic such as phenol or thymol. The use of inorganic antiseptics—particularly salts of heavy metals—was not found to be useful because they reacted with the protein rendering it insoluble. The antiseptic action was also rapidly lost.

In some cases, it was found advantageous to pass the precipitate repeatedly through a hydro-extractor after addition of necessary quantities of kaolin or other suitable binding material. The centrifugal action combined with the draught of air helps not only to remove the surplus quantity of water but also to dry the product to a stage when it can be spread out on the floor without the danger of fermentation setting in.

The dry product thus obtained is first crushed to a coarse condition and then powdered to a fine state of division passing the 80-mesh sieve. It is then suitable for use as basic material for the preparation of paints, plastics, insecticidal sprays and a variety of other products.

For many purposes such as the preparation of water or oil-bound paints or even the preparation of certain plastics the wet paste can be used as such, adding the necessary pigments and oil or fillers as the case may be. The finished product may be either stored as such or dried, powdered and packed as may be required.

The liquid extract left after the separation of protein is transferred into shallow basins prepared by enclosing requisite areas of earthen flooring with brick and clay borders not exceeding 6″ in height. The rate of drying being dependent on a number of factors, it would be difficult to present a definite estimate regarding the area required for treating extracts from large quantities of cake, but it may be stated that, under favourable conditions, a 20′ square would suffice to dry the concentrated extract from a cwt. of cake, in the course of a bright and sunny day. The nature of the floor, the efficiency of the sub-soil drainage as also the direction of wind are important factors determining the rate of drying the extract. As already indicated, the preparation of enclosures for drying the extract requires no big outlay. Even single rows of half-burnt bricks fixed lengthwise and plastered with clay have been found useful for the purpose.

In fine weather, the evaporation goes on fairly rapidly, so there is no danger of any undesirable fermentation setting in during the drying. In cold and humid weather, the drying is slow in spite of the liquid being exposed in very thin layers. Under such conditions it has been found advantageous to add small quantities of finely divided clay to the liquid so that it works up to a paste. In that condition the concentration of the liquid proceeds rapidly. The paste can also be turned over repeatedly to expose fresh surface from time to time and thus hasten the drying.

To the thick paste, thus obtained, the solid residue left after extraction of the protein is added and the mass turned over a few times. During this operation, a considerable portion of the wet paste forms lumps enclosed within comparatively dry powder. Since such lumps are very difficult to dry, they have to be broken up by beating with wooden rods or treading on with wooden shoes, whichever is convenient, so that fresh surfaces are exposed from time to time. The dry product finally obtained is either stored as such or further dried in a current of hot air—if the weather conditions are unfavourable—prior to storage.

The composition of the finished product is nearly the same as that of the original cake; in fact it is richer in soluble nitrogen and minerals so that its use as a manure would appear to be highly promising. Its manurial value as compared with those of other organic manures is now under investigation and will form the subject of a later communication.

UTILISATION OF VEGETABLE CASEIN IN SOME INDUSTRIES.

Water-paints.—A big branch of the paint trade which has lately been increasing in popularity is the manufacture of water-paints. Such paints are in great demand because of their various excellent qualities combined with cheapness. They are also easily handled and require no special equipment to apply them. They are as convenient to handle as white-washes but are very much superior to the latter in quality.

A preparation of water-paint is made up chiefly of (1) a pigment for covering the surface which is usually chalk, (2) a filler to prevent settling and to make the paint work easily (generally white clay is used for this purpose), (3) a colouring material which may be an earth ochre or a dye for tinting the preparation to the desired colour and shade, (4) a substance (usually starch) to ensure that it will not run or the surface, (5) a binder to hold the different materials together and to fix them to the surface to be painted, and (6) an insolubiliser to convert the film of paint, on drying, into a stable entity that will stand washing with water. Of these, the binder is the most important since it determines, more than anything else, the general quality and the durability of the paint. Several substances are being used as binders but most of them are quite unsatisfactory: in fact the ideal binder is yet to be found. To be satisfactory in every respect, the binder must possess the following qualities:—It must have a good grip on the surface to which it is applied. It should adhere readily to a variety of surfaces, and, having stuck, it should withstand rubbing or mechanical pressure. Thus, it should not scrape off even after vigorous rubbing with the hand. The nature of the binder should be such that it would become insoluble either spontaneously or after some inexpensive treatment. Lastly, the binder itself should be very cheap so that the cost of the paint may be maintained as low as possible. These conditions are not however satisfied by the binders present in the commoner brands of water-paints on the market which contain either water-soluble gums or cooked starch The latter, though otherwise good adhesives, do not stand rubbing or washing with water so that the paints containing them are not much superior to white-washes. The better class paints contain either glue together with paraform or hexamethylene tetramine or milk casein with lime as the insolubiliser. Of these, casein paints are decidedly the most satisfactory with regard to both adhesiveness and stability. Even the best grades of glue assisted by the most efficient treatments for water-proofing do not give such satisfactory products as milk casein. Casein is difficult to peptise, however, so that quite a considerable part of that protein remains undissolved even in presence of dilute alkali. Slaked lime is fairly useful in rendering casein partially soluble, but on exposure to air it absorbs carbon dioxide fairly rapidly so that it soon loses that property. As the result of this, many commercial brands of pastes or powders contain very little caustic lime so that the casein, though present in them, does not serve effectively as the binder. Such paint preparations fail in the hands of the consumer.

In view of the similarity in properties between vegetable proteins and milk casein, difficulties similar to those already mentioned were anticipated and attempts made to overcome them. The conditions for the initial peptisation of the protein were carefully standardised so that, on addition of water, the major part of the material was fairly uniformly dispersed. A number of treatments were also studied so as to facilitate the peptised protein becoming insoluble on standing without, in any way, impairing its properties as an adhesive. A number of paint preparations incorporating the different treatments were then produced and their qualities compared with those of different popular brands available on the Indian market.

In many of the commoner water-paints, the main ingredient is chalk, which is used because of its good covering power. Chalk of good quality is not available, however, in many parts of India, so a suitable substitute has to be found. After some trials, it was found that white clay (kaolin), if properly ground, and mixed evenly with the peptised protein gave as satisfactory results as good grades of chalk. The South Indian kaolin, such as is found in certain parts of Mysore and Travancore, possesses good covering power and can be easily tinted to any desired colour or shade. For the latter purpose, earth ochres cheaply available in many parts of the country were found quite useful. Paints possessing certain unusual shades as also certain bright colours cannot, however, be prepared by using earth ochres alone. For this purpose, it was found necessary to use some insoluble inorganic pigments or aniline dyes.

Some experiments were carried out using lime as the insolubiliser* for vegetable casein. After a number of trials, the following combination was found to be most suitable for the purpose :—A number of mixtures each containing 13 parts of basic material containing the protein together with some starch, gypsum and a small amount of kaolin (originally used as the starter), 10 parts of kaolin with ochre or dye (used for tinting) and 5 parts of slaked lime were prepared and ground into pastes with addition of increasing quantities of water until the desired consistency was attained. Preparations of paint obtained in the above manner were then applied on uniform pieces of wood and the quality of the films compared with those of a few well-known brands of water-paints available on the market. It was observed that the vegetable casein paints dried rapidly, leaving smooth films which could not be easily rubbed off. Second coats could also be applied on top of the dried films without working on the under-coats. The finished paints stood rubbing and washing with water and though some pressure was applied, they could not be easily scraped out. On the other hand, the commercial brands did not stand much rubbing; they also tended to wash off with water so that even on gentle rubbing the entire mass of those paints were removed from the surface.

The foregoing tests having indicated that the preparations of paints obtained with vegetable casein as the binder were of good quality,

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* In the present case, the term 'insolubiliser' is rather misleading because the same chemical has also to function as the solubiliser. Neither milk casein nor the vegetable product can dissolve by itself in water. It has to be brought into solution by some alkali. Lime is the cheapest chemical that can be used for that purpose. The insolubilising action of lime comes only after the paint has been applied to a surface. It absorbs atmospheric carbon dioxide turning into insoluble carbonate. It loses its solvent action on the protein which thus reverts to the insoluble condition. These changes are complete within a few days and the dried film is then enabled to stand rubbing and washing with water.

further trials were carried out applying the paints to a variety of surfaces such as walls, stones, and cement slabs and studying the manner in which the paints adhered to the surfaces, filled up cracks and crevices and stood rubbing and washing. On all such surfaces the vegetable casein paints adhered more firmly, covered up the surface and filled up the crevices more effectively than any of the other preparations with which they were compared.

In many European countries, and to some extent in America, the better class water-paints, particularly those containing casein, are marketed as ready mixed pastes which can be diluted with water to the desired consistency. Such paints, while possessing several advantages, particularly with regard to ease of mixing and high adhesive power, over the dry powder are yet too costly for the average consumer. This is chiefly due to the presence of water which increases the weight of the preparation and the need for using special metallic containers which may sometimes cost even more than the paints. In view of the above and the desirability of producing a preparation which can be mixed with water or oil at the will of the user, some experiments were carried out drying the pastes and storing them as fine, dry powders in paper containers. The drying was carried out at different temperatures with and without using a draft of air to carry away the water vapour. Trials with the powders thus obtained showed, however, although the paints were highly satisfactory as wet pastes, the dry products could not be readily brought into homogeneous suspension: nor did they adhere properly to the surfaces to which they were applied. The dried films came off very easily with rubbing. They were also easily washed away by water. These observations suggested that, during the drying, the binder (the protein) had lost its adhesive property so that the paints obtained from the dry powder were no more than white-washes. It is this difficulty, probably, which precludes the marketing of good quality casein paints as dry powders.

With a view to overcoming this defect, a number of experiments were conducted, adding to the dry paint mixtures different substances which would help to peptise the vegetable casein on wetting with water. Since slaked lime proved unsatisfactory, the use of other chemicals was tried. Caustic alkali and alkali carbonate were both found useful, but the carbonate was found to be more convenient to handle because of its non-hygroscopic character. A number of concentrations of the carbonate were tried as the result of which it was found that 2·0–2·5 per cent. on the weight of the dry paint powder was quite adequate for the purpose.

The following would be a convenient procedure for the preparation of dry paint containing alkali carbonate. The dried protein preparation containing small quantities of starch and gypsum is intimately mixed with the required quantity of sodium carbonate and the mixture ground to fine powder. In this manner, the intimate mixture of the protein and the alkali carbonate would first be ensured. Kaolin is next added and the grinding continued until the mixture forms a homogeneous product. The pigment or mixture of pigments as the case may be, are finally added in quantities necessary to produce the desired colour and shade. The dried powder thus prepared can be kept indefinitely without undergoing any appreciable change. On addition of water, however, the carbonate first dissolves and acts on the protein bringing it into solution. After standing for a few minutes the major part of the finely divided protein passes into solution and the paint is ready for application. By repeated stirring, the peptised protein comes into contact with all the components of the paint and is thus enabled to hold them firmly on setting.

It was observed that the paint prepared in the above manner was rather slow in setting to an insoluble condition. That process can be hastened, however, by adding a small amount of burnt or slaked lime to the alkali carbonate. The carbonate of lime is formed rapidly and thus causes the protein to coagulate more rapidly than might otherwise be the case.

Oil-bound paints.—There is considerable demand for cheap paint materials that would be suitable for covering a variety of surfaces, protecting them against the effects of adverse weather, ravages of insects and fungi, and, at the same time, possessing the water-proofness and other good qualities which characterise the superior classes of oil-paints. The commoner oil-paints are rather costly and consume large quantities of oil which are required not only for the preparation of the paste but also for dilution to the required consistency. Moreover, oil-paints are generally supplied only in the form of ready-mixed pastes which though possessing certain advantages, yet involve the use of special metallic containers. In view of the above, some experiments were carried out preparing oil-bound water-paints with vegetable casein as the binder. As the result of these trials it was found that although the oil could be worked up with the paint material in any proportion, it was not generally possible to dilute such preparations with water without the separation of oil. It was also noted that there is a certain optimum proportion which should be maintained between the quantities of protein, water and oil used in the initial mixture for facilitating indefinite dilution with water at a later stage. The proportion of sodium carbonate to protein preparation also had a decided influence on the suitability of the paint emulsion to stand dilution with water. If the carbonate was below the optimum percentage, the oil tended to separate from the paint thus rendering the distribution inadequate. After a number of trials it was found that a satisfactory procedure would be to first grind up the paint mixture (wet paste) with increasing quantities of oil until a smooth paste could be obtained. A small nucleus of paste containing useful amounts of soap would first be formed which, together with the free carbonate left unused, would soon help to emulsify further quantities of oil upto a maximum which can be determined in each case by actual trial. The oil-bound paste thus obtained would then remain stable and stand dilution with water without any of the oil separating. The diluted paint could also be applied to any surface ensuring satisfactory covering, colour and water-proofness which are characteristic of oil-paints.

It does not appear to be feasible, however, to prepare a dry paint mixture to which indefinite quantities of oil and water can be added. The chief difficulty is the emulsification of the oil which could not be satisfactorily carried out by casual grinding in an ordinary mortar. Careful mixing of the oil, water and alkali followed by prolonged grinding in power-driven mixer is necessary to ensure a good paint mixture which would stand indefinite dilution with water. Further work is in progress to determine whether any of the other emulsifiers could help to bind together the oil, protein and water in different proportions without having to resort to prolonged grinding as in the case of soda. Attempts are also being made to prepare thick emulsions of oil which could be added in any desired quantity to the dry paint powder and could be diluted to any required consistency by mere addition of water. In this manner a dry paint mixture suitable for use either as a water paint or as an oil-bound water paint can be supplied to consumers.

Use of vegetable casein as spreader for insecticidal sprays.—In recent years, the use of chemicals as sprays for the control of insect or weed pests and fungus disease has become increasingly popular. In tropical countries, the spraying of coffee, areca palm and fruit trees has become almost a regular field operation. In actual practice, however, the farmer does not get the full benefit of the sprayed chemical because the latter (*a*) does not, by itself, possess good covering power, (*b*) has no adhesive properties, and (*c*) has the tendency to flake and peel off or get washed out by rain. As the result of this, the spraying operation has to be repeated several times in a season if the pests are to be effectively kept out. The cost of spraying is thus greatly increased without adequate return, so that the need for a cheap spreader that would help to overcome the above defects is now generally recognised.

The possible use of several chemicals as spreaders has been indicated but most of them have proved unsatisfactory in practice. Robinson compared the covering qualities of a number of spreaders and found that proteins were the most efficient, at the lowest concentrations, for the largest number of surfaces tested by him (*J. Agric. Res.*, 1925, 31, 71). Milk casein is known to be fairly efficient and in view of its similarity in properties to vegetable casein and the excellent adhesive quality of the latter, some experiments were carried out using the vegetable casein as spreader for insecticidal sprays. Since the preparation of protein from Hongay seed-cake also contains small quantities of the bitter principle with which the original cake is associated, it was considered that the insecticidal action of the spray would be augmented by the use of that protein.

One part of the crude vegetable casein preparation (Nitrogen 5·6 per cent.) together with 2 parts of copper sulphate (hydrated) and one part of burnt lime were ground together with sufficient quantity of water until a homogeneous paste was obtained. More water was then added, with frequent stirring, to dilute it to a thin suspension corresponding, in strength, to that ordinarily used in field practice. The spray solution thus prepared was compared with standard Bordeaux mixture with regard to both adhesiveness and washability.

The preliminary test consisted in applying uniform drops of the suspensions to the surface of similar pieces of wood and measuring the areas covered by them. After the films had dried the wood pieces were placed under a flowing stream of water and rubbed with the hand to compare their ability to stand the treatment. It was observed that the spots of standard Bordeaux mixture solution (without the spreader) dried after some hours forming relatively narrow circlets which could be easily removed by gentle rubbing. When placed under water, those films washed off readily even without application of pressure. On the other hand, the spots containing vegetable casein dried forming thin and wide circles which adhered firmly to the surface and could not be scraped out by mere rubbing. The films containing the protein also stood washing and could not be removed even after rubbing under water. These observations indicated that the vegetable casein would make a highly useful spreader-adhesive for insecticidal sprays.

Some field trials were next carried out, spraying different kinds of vegetation with suspensions of Bordeaux mixture prepared with and without vegetable casein. It was noted that the plants sprayed with the former were dotted dark blue-green while the latter developed the characteristic light blue spots ordinarily associated with Bordeaux mixture. Even after a few days of drying in the sun, the mixture without the adhesive tended to flake and drop off while no such tendency was observed in the case of the preparation containing vegetable casein. The showers which followed in a few days washed away the little amount of the Bordeaux mixture prepared without the adhesive. On the other hand, the plants sprayed with the suspension containing the protein still carried the entire amount of the spray mixture so that a repetition of the operation was obviated. The heavy rains and the humid weather which succeeded helped further to demonstrate the excellent qualities of the adhesive. Even at the time of writing this paper (six months after the spraying) the insecticide is still in tact on the plants so that further spraying has not been necessary. These results leave no doubt regarding the superior quality of the vegetable casein as a spreader-adhesive.

Utilisation of vegetable casein in the plastic industry.—During recent years, the plastic industry has been gaining considerably in importance and various articles of utility as well as beauty are being manufactured out of natural as well as synthetic products now available on the market. Milk casein is one of the more important substances used in the plastic industry and a large number and variety of articles such as buttons, brushes, combs, umbrella and knife handles, dolls and toys are being manufactured out of it. The world's supply of milk casein is not, however, unlimited so that the possibilities of extending its application are naturally restricted. On the other hand, the vast abundance and relative cheapness of the vegetable product would suggest the possibility of its being not only a supplement but also a useful substitute for milk casein. Its remarkable adhesive properties are already well established. Evidence has also been obtained to show that it sets even at ordinary temperature without much application of pressure. There is no perceptible shrinkage in volume on drying. The finished product is water-proof, non-explosive and non-combustible. These observations would suggest that the vegetable casein has immense possibilities of application in the plastic industry.

Further work is in progress standardising the conditions for the production of vegetable casein on a large scale. Attempts are being made to reduce the cost of the different operations involved and to avoid undue waste of protein and other valuable constituents present in the seed-cake. It is not possible at this stage to present a detailed estimate for the working of a small factory, but small-scale trials with quantities of cake upto a hundredweight of cake have shown that even if the materials used for the preparation of protein are purchased on retail basis, the cost of production of the dried product would be under half an anna (approximately ½*d.*) per pound. It is expected that production on a bigger scale and using cheaper raw materials would further reduce the cost. In view of the abundance of raw material and the low cost of manufacture, it would appear that the vegetable casein has a great chance of standing competition not only with milk casein but also other materials used for similar purpose in the paint or plastic industry.

The processes relating to the manufacture of vegetable casein by the use of gypsum or the biochemical method as also its application in the paint industry and the preparation of insecticidal sprays have been covered by Patents (Government of India, Patent Specification Nos. 20143, 20192, 20193). It is hoped that, with the processes fully standardised, the manufacture of vegetable casein and its use in different industries would prove to be a highly profitable source of business for those interested in the venture.

SUMMARY.

1. The present position with regard to seed-cakes has been discussed and the need for finding new uses, particularly the preparation of vegetable casein, indicated.

2. Among the different solvents that were tried, alkali carbonate was found to be the most satisfactory for the extraction of protein from Hongay seed-cake.

3. The conditions for the extraction of protein have been standardised. Coarsely powdered cake (200 parts) are treated with minimum quantity of alkali carbonate (3 parts) for the complete extraction. The extract is separated by either passing the suspension through a close meshed sieve or, preferably, rotating in a hydro-extractor. The small quantities of starch passing into the extract can be separated by centrifuging.

4. Precipitation of the protein by treatment with mineral acids has been found to be not only wasteful but also unsatisfactory. Higher yield as also better product was obtained by treating the alkali extract with gypsum, kaolin or mixture of gypsum and kaolin and allowing the suspension to stand for 2 days. Neutralisation of the alkali as also the separation of the protein were greatly facilitated by the organic acids and carbon dioxide resulting from the fermentation which accompanied the treatment. The details of the related operations as also the drying of the residual solid and supernatant liquid have been worked out.

5. The nitrogen balances at different stages during the precipitation of the protein and the preparation of the manure have been determined. Non-protein nitrogen, equivalent to 16 per cent. of the nitrogen present in the original cake, is lost during the fermentation preceding the separation of the protein.

6. The conditions for the preparation of water-paints and oil-bound water-paints containing vegetable casein as the binder have been standardised. A number of trials carried out with the products show that they possess excellent covering and adhesive properties, can withstand weathering and are quite washable.

7. Laboratory tests, as also field trials, have shown that the vegetable casein makes a good spreader-adhesive for insecticidal sprays.

8. The possibilities of using vegetable casein in the plastic industry are indicated.

Department of Biochemistry,
Indian Institute of Science,
Bangalore.

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