LARGE-SCALE EXTRACTION OF CEREAL BIOPOLYMERS

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Cereal grains can be considered as an agricultural raw material rich in several biopolymers. The major biopolymers are starch, protein, non-starch polysaccharides and lipids. Primary extraction of the biopolymers normally involves dry or wet milling, or a combination of the two. Conventional dry milling primarily separates the grain into its anatomical components, which are to some extent enriched in certain biopolymers, for example endosperm flour is approximately 80% starch. Further enrichment of particular polymers can be achieved on the basis of density through air-classification. Wet milling, which also involves size separation by sieving and density separation by hydroclones and centrifugation, is particular effective at separating and purifying the individual biopolymers.

Improvements in the efficiency of extraction of individual biopolymers may be achieved by several strategies. Co-products of food processing may be particularly rich in a certain biopolymer, for example hominy chop from maize dry milling is a valuable source of oil. Solvents that specifically extract specific biopolymers may be used, for example aqueous-alcohol to extract the prolamin proteins. Commercial hydrolytic enzymes, such as hemicellulases and amylases, can be used to purify crude protein extracts by removal of contaminating polysaccharides. Solvent recovery and reduction in energy costs are also crucial to the viability of extraction.

INTRODUCTION

Cereal grains can be considered as an agricultural raw material rich in several different natural polymers (biopolymers)[1]. The commercial extraction of biopolymers from cereal grains has a long history. Wet milling of maize for starch dates back to the mid 19th century. The first patent for extraction of zein, the maize prolamin protein, was granted to the pioneer cereal chemist T.B. Osborne in 1891[2]. Today, wet milling of maize is a very large industry, around 40 million tonnes per year. However, sorghum and millet have up until now hardly been exploited. This is notwithstanding the fact that for several years has been a resurgence of interest in extraction of biopolymers from cereal grains that has been driven by factors such as:

Cereal grain overproduction and low prices,

Consumer demand for renewable, natural and biodegradable alternatives to synthetic polymers from the petrochemical industry,

Governmental environmental protection legislation to reduce waste streams from both processing industries and the end-user,

Increasing cereal co-product production from grain fuel ethanol plants, particularly in the USA.

In decreasing order of quantity, the biopolymers of cereals grains are:

Starch (approx. 70%) - two types of molecules: amylopectin (branched chained) and amylose (essentially straight chained),

Proteins of various types (approx. 10-12%) – the aqueous alcohol soluble prolamin group accounting for some 60% of the protein in most cereal grains (it should be noted, however, that prolamins themselves are very varied group of proteins, with widely differing functional properties),

Non-starch polysaccharides (dietary fibre) (approx. 9-12%) of various types - including cellulose, mixed linkage beta-glucans and pentosans (mainly xylans),

Lipids (approx. 3-5%) – mainly triacylglycerols (triglycerides).

This paper will examine technologies that are and can be applied commercially to extract, separate and purify these cereal grain biopolymers. Factors affecting efficiency of extraction will be highlighted. The focus will be on sorghum but with frequent reference to maize, since these two cereal grains are very similar in structure and composition and there is an enormous literature on maize milling.

MILLING

Milling is the primary process that is used to extract the biopolymers from cereal grains. Milling is a very general term, covering a huge range of technologies that separate the grain into its anatomical and chemical components as well as simply grinding the grain into small particles. Milling technologies can be classified into dry milling (milling in air) and wet milling (milling in water). However, it must be emphasised that even in dry milling, moisture is often applied to the grain to aid in its dissolution.

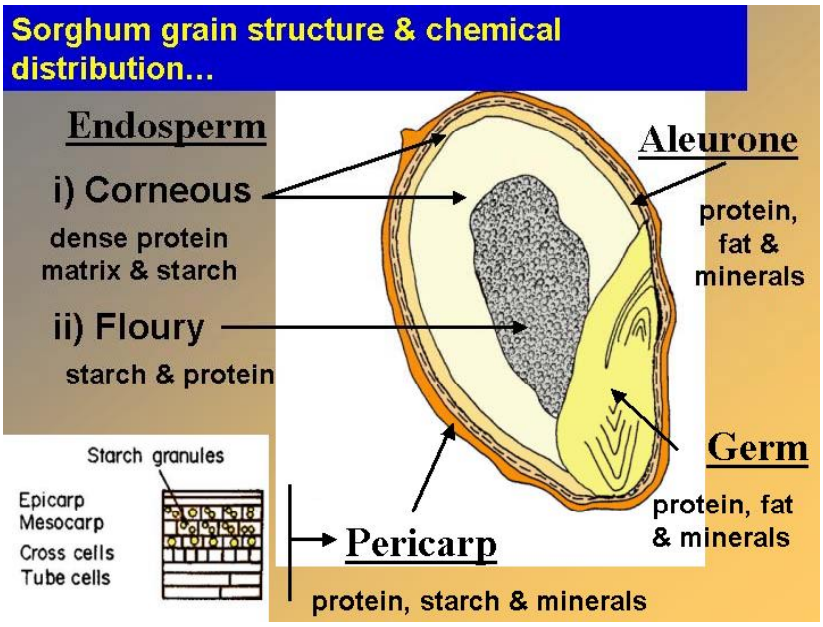


Figure 1 Components of sorghum grain and distribution of biopolymers

Dry milling

Dry milling technologies aim primarily to separate the cereal grain into its anatomical components: pericarp (bran), germ and endosperm, and to grind the latter into meal or flour.

Since the polymers of the cereal grain are not evenly distributed in the grain (Fig. 1), some purification is achieved. For example, the pericarp is very rich in non-starch polysaccharides, while the lipids are concentrated in the germ. Separation of the anatomical components in dry milling is achieved by technologies such as abrading the pericarp off the grain and using impact milling to break the germ away from the endosperm. Using these two techniques in sequence (so-called integrated process) high separation efficiency can be achieved with sorghum grain (Table I). The anatomical components are purified on the basis of size and shape by sieving and "density" by aspiration and air-floatation. These techniques generally separate the dense small particles of endosperm from the large less dense pieces of bran and germ. Some separation of starch and protein in the endosperm can be achieved by "air-classification", following fine grinding.

Milling fraction	**Yield (%)**	**Moisture (%)**	**Crude fat (%)**	**Crude fibre (%)**
Large grits	50	5.9	0.8	0.2
Small grits	23	5.9	0.5	0.2
Flour	4	7.3	3.0	0.9
Germ	7	5.2	15.0	0
Bran	16	8.1	9.0	7.8

Table I Products of integrated dry milling of sorghum grain (adapted from Rooney[3])

Wet milling

In contrast to dry milling, the primary aim of wet milling is to separate and extract the grain biopolymers. The medium of water allows much more fine milling of the grain as heat generation through friction is greatly reduced, and freeing the starch granules from their protein matrix. Water also enables much better suspension of individual particles than air, facilitating their separation on the basis of density. Figure 2 shows the wet milling process that has been used commercially for sorghum. The critical step is steeping the grain.

Steeping hydrates and softens the grain. This enables a clean separation of the germ from the endosperm and weakens the bond between the starch granules and protein matrix, allowing their separation. Sulphur dioxide is included in the steep water and lactic acid is produced lactic acid bacterial fermentation during steeping. It is well established that the sulphur dioxide breaks disulphide bonds in the prolamin proteins softening the protein matrix[5]. The lactic acid also has a protein softening effect, although the exact mechanism is disputed.

In wet milling a key technology is the hydroclone (Fig. 3). This is a sort of "water cyclone", separating particles on the basis of density through a swirling action. Hydroclones are used to separate the fat-rich, less dense germ from the rest of the grain and to purify the dense starch granules. Other separations, such as fibre from starch and protein, and starch from protein are achieved by wet sieving and centrifugation, respectively.

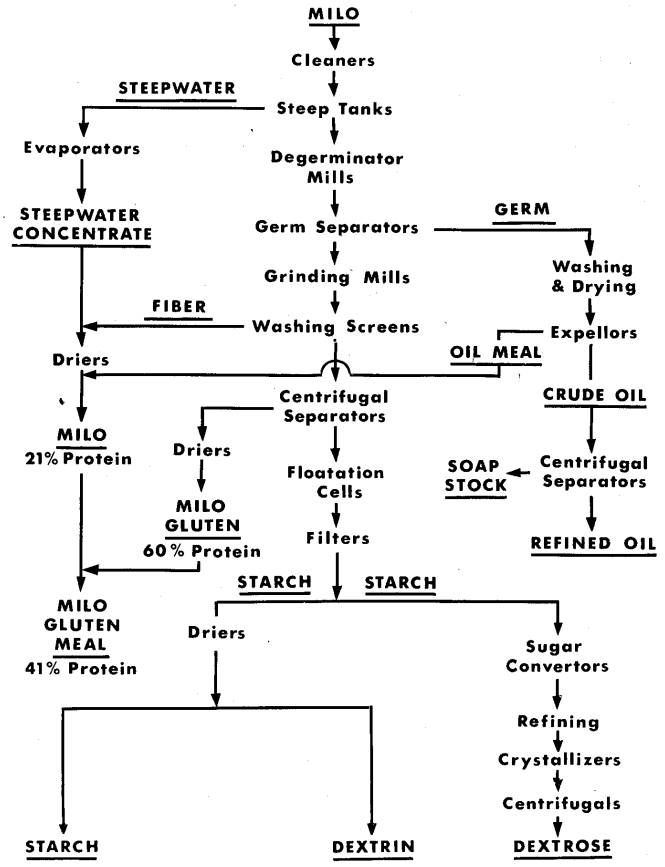
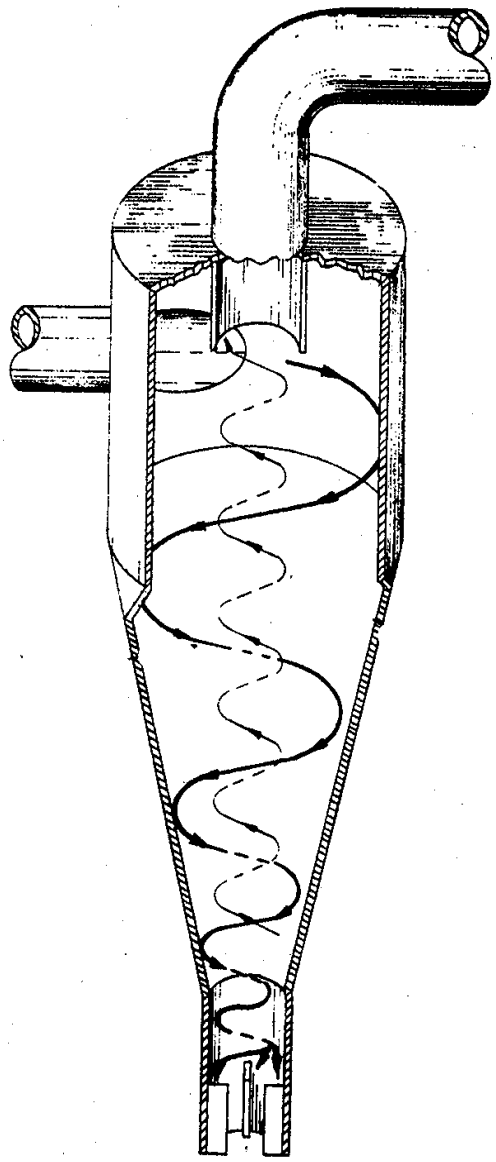


Figure 2 Flowchart for sorghum industrial wet milling (from Watson[4])



Solvent extraction processes

Figure 3 Operation of a hydroclone (from Watson[4])

In general extraction is the separation of a liquid or solid substance from a mixture of other solids and liquids by means of a selective solvent, in which the preferred substance is more soluble than all the others[6]. The operation in which we are interested, the extraction of cereal biopolymers, is referred to as "leaching" or "lixiviation". This is defined as the removal of a soluble fraction from an insoluble

permeable solid phase. The separation involves selective dissolution or washing out of the soluble constituent[7].

Leaching is commonly known as a chemical engineering process, but in the food industry the most well-known application area is in the field of oil extraction[6]. Effective leaching requires a number of parameters that need optimisation in order to design a profitable process[7]. These parameters are[7]:

- Choice of solvent – must offer the best balance between high saturation limit, selectivity to the solid being extracted, capability to produce extracted material of quality unimpaired by the solvent, chemical stability under process conditions, low viscosity, low vapour pressure, low toxicity and flammability, low density, low surface tension, ease and economy of recovery from the extract stream and price
- Temperature – must be chosen to offer the best balance of solubility, solvent-vapour pressure, solute diffusivity, solvent selectivity and sensitivity of product
- Type of reactor – select the reactor that is most compatible with the desired process (reliability and profitability).

The ideal solvents should have the following characteristics[6,7]:

- A narrow and not too high boiling point or range, should remain liquid even at very low temperatures
- Neutral to the solid being extracted and must dissolve the solid easily and selectively
- Be stable and inert when in contact with metal surfaces
- Low specific heat, low heat of evaporation and a low viscosity and density
- Non-toxic
- Preferably inflammable and non-explosive
- Available at low prices and in adequate quantities.

There is no solvent that will fulfil all these criteria. Solvents are selected for practical purposes suited to the material handling requirements.

The five systems that are most often used for seed-tissue leaching operations are described below [6,7].

Single-stage batch contact

In this system, the fresh substrate (flakes or crumbs) and the fresh solvent are contacted in a kettle ("pot" or batch contactor). Whether the two phases are mixed or not, after arriving at equilibrium, the solvent containing the dissolved solid is removed and the solvent is distilled off. This method is the closest system to the laboratory conditions typically referred to in the extraction of cereal polymers on small scale[8-10]. This operation is not considered very effective as large amounts of fresh solvent are required and final solutions are very dilute. Batch extractors are generally cylindrical pots of 2–10 m^3 volume and can be installed horizontally or vertically. They are provided with built-in filter bottoms or separators. These extractors have no internal

mixing devices when they are vertical, but may have rotary or oscillatory mixers if placed horizontally. They may have heated jackets and they are filled by gravity.

Co-current multiple-stage single-batch contact

This method is employed in the laboratory Soxhlet extractors and in some industrial batch systems. The first volume of solvent is drawn off after equilibrium has been attained and a second fresh portion of solvent is added. The process is repeated a few times. Every portion of drawn-off solution is continuously being distilled in order to reuse the solvent immediately. The disadvantage of this system is that the final solution is very dilute and the cost of distillation is prohibitive[6].

Counter-current multiple-stage single-batch contact

A more efficient version than a co-current system is a counter current system. First, a nearly saturated solution is added to the fresh substrate in a rotating extractor. After withdrawal, the solution is distilled. The second contact is done in the same extractor using a less concentrated solution and the last contact is done using fresh solvent. All the extractions are done in one extractor ("batch").

Counter-current (multiple stage) multi-batch contact

This system uses at least four interconnected batch extractors with the fresh substrate placed in the fourth one where they are contacted with linear solvent coming from the third extractor. The third extractor obtains its solvent from the second and the second obtains its solvent from the first where almost clean substrate is brought into contact with fresh solvent. The fresh solvent is able to remove residual components out of the substrate, while the almost saturated solvent can still remove more components out of fresh substrate.

Counter-current multiple stage true continuous contact

In all the previous systems, the substrate is static and the solvent is moving. If the substrate is also moving in one direction and the solvent in another, the operation is called the counter-current multiple stage true continuous contact system and is proven to be the most economic. In practical operations, the percolation principle of the multi-stage batch contact systems as described is used, or the substrate is moved through the system by totally immersing them in solvent.

Various designs of continuous extractors exist such as immersion extractors, percolation extractors, Vertical basket ("Bollmann") extractors, horizontal basket types, the horizontal rotary extractor and horizontal belt-type percolation extractors.

Solvent extraction of cereal proteins

Cereal protein and cereal oils are the two polymers obtained by using solvent extraction[11,12]. Maize and kafirin proteins, which are the prolamin storage proteins, are soluble in aqueous alcohol plus a reducing agent. Kafirin proteins are not extracted industrially, but various laboratory processes exist[8-10]. These systems have

been optimised to a large extent, but unfortunately, the best solvent is 60% tertiary butyl alcohol and 0.05% dithiothreitol (a reducing agent), which, although suitable for academic studies, is not food compatible. Kafirin can be extracted in 70% aqueous ethanol and a reducing agent, but only at 70ºC, which does have a potential damaging effect to the protein.

Zein has been extracted industrially since 1939 using a two-solvent process. The extraction solvent consists of hot aqueous isopropyl alcohol (86%)[13]. Currently, zein is being manufactured by a patented process using aqueous isopropyl alcohol or aqueous ethanol[13,14]. In this process, maize gluten is extracted at 60ºC with 88% aqueous isopropyl alcohol containing 0.25% sodium hydroxide and the extract separated centrifugally. The clarified extract is then chilled to -15ºC causing the zein to precipitate as a "taffy"-like layer. The supernatant is decanted and the lower layer, containing 30% zein, is dried on a vacuum drum drier or a flash dryer. A purer product is made by re-dissolving and re-precipitating the zein. Yields are 20–24% based on corn (maize) gluten. Re-precipitation can also be done using aqueous ethanol[13].

In the case of extracting cereal proteins, solvents used include isopropanol and aqueous ethanol[8]. Zein is known to be also soluble in propylene glycol and aqueous ammonia[15]. It is sold as a powder or a solution for ready-to-coat applications. For the zein process, research has been done in the field of reduced temperature extraction systems in order to reduce heat-induced damage.

In order for the industrial-scale extraction of sorghum kafirin and other cereal proteins to be implemented efficiently for use in the food industry, solvents must be food compatible and easy to handle. At this stage, aqueous ethanol extraction of kafirin is the most practical.

IMPROVING BIOPOLYMER EXTRACTION VIABILITY

Improvement in the economic viability of extracting cereal grain biopolymers is the subject of ongoing research worldwide. For example, it has been a major focus of the United States Department of Agriculture's (USDA) Northern Regional Laboratory (now National Center for Agriculture Utilization Research) at Peoria, Illinois since its inception in the 1940[16].

Raw materials for extraction

A major way of improving the efficiency of biopolymer extraction is by the use of materials for extraction that are already enriched with the biopolymer(s) of interest. For example, degermination in maize dry milling produces a co-product referred to as "hominy chop" which comprises the maize grain hull (pericarp) and germ. The former is rich in fibre and the latter rich in lipids. In brewing and fuel ethanol production the grain starch is solubilised and hydrolysed leaving the co-product known as "spent grain" or "distillers grain", which is a very considerably enriched source of protein, fibre and lipids (Table II).

Chemical component	Whole grain (%)	After removal of starch and simple sugars (%)
Starch	73.4	0
Simple sugars	1.9	0
Protein	9.1	36.8a,b
Fat	4.4	17.8a
Non-starch polysaccharides	9.8	39.7a
Ash	1.4	5.7a

aA proportion of this component would be water-soluble, thus the actual percentage remaining may be lower
bActual commercial corn distillers grain data 27.1-29.5% protein[8]

Table II Change in the proximate composition (dry basis) of maize grain as a result of removal of starch and simple sugars as would occur in brewing and fuel ethanol production (adapted fromWatson[18])

Improvements in process

An important issue with regard to process efficiency concerning sorghum grain specifically is the nature of its pericarp. The sorghum pericarp is somewhat friable and during dry milling easily breaks into small pieces that contaminate the flour. Various methods have been investigated to remove the sorghum pericarp more completely. There have been patents and now recently a paper on removal of the sorghum and pearl millet pericarp by treatment with alkali[18]. We have achieved some success experimentally in loosening the pericarp using commercial cellulase enzymes[19]. A problem in wet milling is that the anthocyanin and anthocyanidin pigments in the pericarp of many sorghum varieties stain the starch[20]. It this regard, it is mentioned in a text on sorghum wet milling that when the grain has been steeped the pericarp can be readily removed with a rubber-covered impeller[4].

In maize wet milling, research and development has been undertaken to reduce or eliminate the use of sulphur dioxide in the steep[5]. A process of steeping under pressure has been developed. However, in view of the fact that sorghum kafirins are heavily cross-linked it seems unlikely that this will be very effective with sorghum. As an alternative, steeping with commercial enzymes has been investigated as means of aiding the separation the biopolymers. Some success in improving maize starch purity has achieved by steeping with the addition of commercial proteinase enzymes[5]. However, this obviously is a “double-edged sword” since the quality of the protein co-product will be adversely affected by such a treatment.

Of more potential is the application of commercial hemicellulolytic, or more specifically pentosanase/xylanase, enzymes. In recent years there has been a dramatic increase in our understanding of the nature of these enzymes and their pentosan cereal grain cell wall substrates[21]. The commercial exploitation of these pentosanase enzymes has revolutionised commercial breadmaking leading to increased loaf volume, softness and shelf-life. The application of hemicellulolytic enzymes to improve sorghum wet milling efficiency has been investigated. Serna-Saldivar and co-workers in 1997 reported on the application of a commercial beta-glucanase[22]. Perhaps not surprising in view of the choice of enzyme, little improvement occurred. However, very recently the same group is reporting much better success using a more

appropriate cell wall degrading enzyme system[23], a reflection of improved understanding of these enzymes and their substrates. With regard to this workshop's emphasis on protein extraction, the use of commercial amylases to purify the protein preparations is obviously feasible.

Value-addition to biopolymers

A critical issue involved in making the extraction of biopolymers more economically feasible is to add value to the biopolymers themselves.

Chemical modification of starches to optimise their properties for different application is huge industry. This is illustrated by the fact that one of the major starch suppliers markets at least 50 different corn (maize) starches. Modifications include: hydrolysis to dextrins and sugars, acid thinning, bleaching, oxidation, cross-linking, derivatisation, chemical substitution, pre-gelatinisation and using starch as a substrate to produce other chemicals[1,24].

Non-starch polysaccharides were historically considered as simply roughage or crude fibre and consigned as a component in ruminant animal feed. However, with our increasing knowledge of the human nutritional importance of dietary fibre, value-addition has taken place. USDA scientists, through a simple treatment of fibre rich cereal co-products with alkali, have developed several types of dietary fibre products, including Oatrim® (soluble fibre from oats), "fluffy cellulose" fibre gel from maize hulls[16,25].

The lipids of cereal grains are mainly polyunsaturated triglycerides and thus a valuable co-product in themselves. A very recent paper on sorghum reports that there are potentially even more valuable "nutraceutical" lipid components called phytosterols in sorghum oil that could be exploited[26].

The proteins of sorghum and millet are the subject of this workshop and covered in depth in many of the other papers. Thus, protein value-addition will not be dealt in detail with here. However, two recent reviews on the processing and uses of zein are of particular interest[2,27]. Applications as diverse as a replacement for shellac in lacquers and varnishes, a binder for cork, a water-resistant coating for paper, in printing inks and photographic emulsions, as a textile fabric, in pharmaceutical tablets and as coatings and films to extend the shelf-life of food products are described.

The viability of zein extraction

In spite of the fact that zein was widely used in the early 1950's as a polymeric substrate for films and fibres, it was too expensive to compete with nylon and polyester. Cost has held back the wider use of zein and currently, the market for pure zein is about 1 million $ per year. Commercially extracted zein costs about 22$ per kg. One of the main reasons for the high cost problem is that very little research has been done on actually optimising zein extraction processes in combination with other cereal extraction systems such as dry milling. A project has recently been initiated by the USDA to re-investigate the economic feasibility of zein extraction in combination with ethanol production plants (by fermentation) and dry milling. Ethanol is produced from the starch in the maize. It was found that by first extracting the zein

with ethanol obtained from the process will greatly increase the efficiency of the fermentation of the starch left behind after extraction. The two processes will therefore work in synergy with each other thereby reducing costs. As the solvent produced by fermentation is the same as the one used for the extraction, equipment for recovery of the ethanol is already available and economies of scale can be achieved in the recovery of dilute ethanol. The substrate is, thus, milled maize meal instead of the traditional gluten feed from the wet milling industry[28]. The process described in Figure 4 shows the extraction of zein with ethanol from a substrate obtained from dry milling. By separation of vitreous and opaque endosperm, the yield of zein can also be optimised as the opaque fraction contains contain almost twice as much zein overall than the vitreous fraction[28]. Extraction rate is also greatly improved by reducing the particle size of the maize flour. However, even with optimisation using dry milling, ethanol recovery after extraction will still be the major cost element necessitating combining the process with an existing ethanol fermentation plant in order to increase economic feasibility.

Work is also being undertaken on the development of low-cost separation systems. After extraction of the zein, the thorough separation of the solvent from the substrate is necessary in order to have a cost-effective process. Success was recently achieved by developing a gravitational settling process into water, which proved to be significantly less costly than both packed bed displacement and centrifugation with rinsing[29].

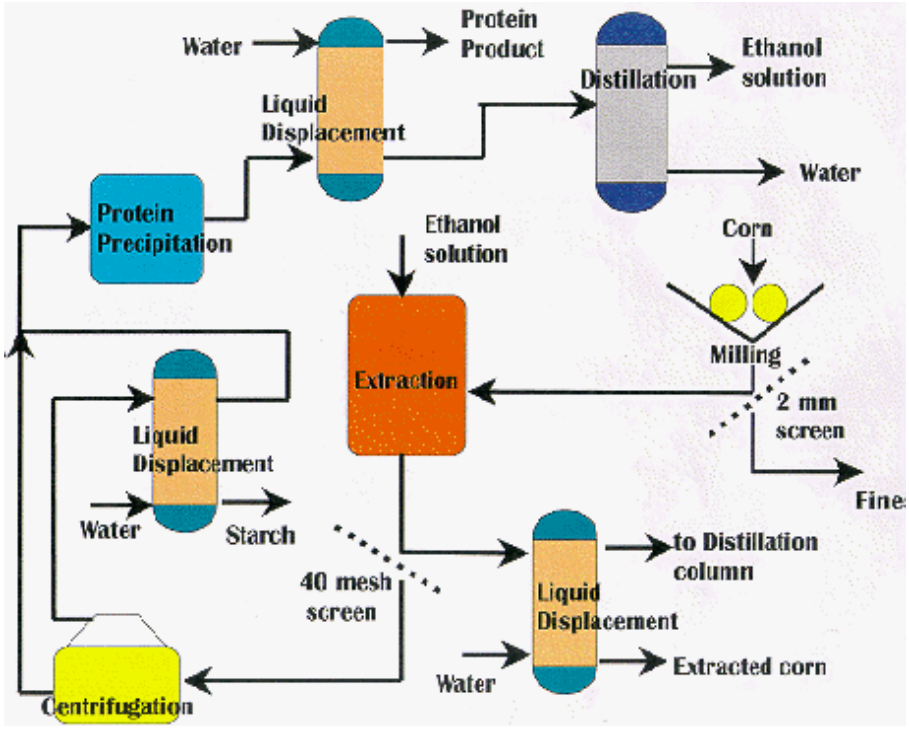


Figure 4 Process for the extraction of zein from maize flour (from Dickey et al[28])

Another important aspect involves the required quality of the end product. Zein for the pharmaceutical industry is highly purified with very small amounts of contaminants such as maize oil. This also increases extraction costs. It is proposed to also investigate end-use specifications as it was suggested that zein films still

containing oil can be of acceptable quality for applications such as films for fast food packages, disposable diapers, tablecloths and bedsheets (providing water resistancy to these materials). Good films can still be made with the oil containing zein, although the films are not of the same transparency – which is not necessary in certain applications. By matching the desired end-use quality with the extraction method, less expensive processes can be developed[30].

CONCLUSIONS

Commercial extraction of biopolymers from sorghum and millet grains will have to be a highly integrated process if it is to be successful. The raw material should be carefully chosen for maximum quantity of the desired biopolymer(s). The processes of biopolymer separation and purification must also be optimised for efficiency. Perhaps most importantly, added-value modifications and applications for the biopolymers should be developed.

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