



**A Return to Natural - The Elimination of  
Solvent Extraction from the Production of  
Oilseed Protein Isolates.**

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David T. Balke PhD

## **A Return to Natural – The Elimination of Solvent Extraction from the Production of Oilseed Protein Isolates**

**At one time, all oil seeds were processed only through natural processes: without the use of solvents. Now, solvents such as hexane are used almost universally in processing soy and other oilseeds. BioExx Specialty Proteins has pioneered a new technology that is able to process oilseeds into food-grade oil and proteins naturally, without the use of noxious solvents, and is now producing food-grade Canola/Rapeseed protein isolates based on a patent pending and proprietary technology using cold pressing and an enzyme-assisted aqueous process. The new proteins retain the full functionality and solubility of the native protein.**

### **Market Demand is Driving Change in Processing Technologies**

With the advent of broad-based marketing of Natural, Non-GMO and Organic certified products, it becomes even more crucial to consider the implications of processing technologies. The use of solvents is a significant issue in the area of natural products and organic production as USDA Organic specification forbids the use of solvents such as hexane. In Canada, the definition of Natural implies minimal processing. Any producer that markets extensively into the natural or organic markets should be aware of the limitations. Even after the extensive processing that hexane-processed oil undergoes, residual levels in the oil have been shown to be as high as 21 ppm. While fully processed isolates are unlikely to have any residual solvent, less processed de-fatted soy flours may have some residual levels.

### **The New Generation of Isolated Proteins**

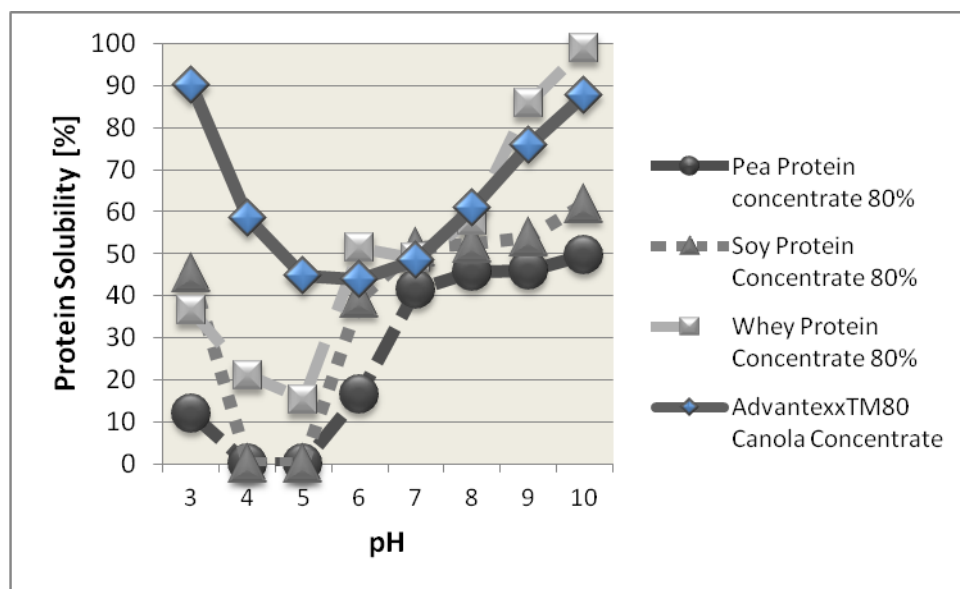
The elimination of solvents has been a dream of processors for years, but most have been handcuffed by use of existing facilities and technologies. Aqueous extraction, which uses a water-based solution to dissolve or wash out material from the seed, has long promised a solution. However, it has not been economically feasible on lower fat oilseeds such as soy. Aqueous extraction is easiest with seeds that don't contain oil. Recovery of the oil from full-fat seed requires dissolution of all of the rest of the seed and then recovery of the oil as a separate phase. This can be very challenging compared to the efficiency of solvent extraction. Loss of the oil value from soy drastically impacts the process economics. One processor in the US has been attempting such a process (Green Planet Farms). In contrast, high oil seeds such as canola(double-zero rapeseed) can generate a substantial amount of revenue from pressing of the seed allowing aqueous processing to be more economical.

From the outset, BioExx has worked towards the highest sustainability and minimum environmental impacts during production of oil and protein from oilseed. The company's most recent advance has been the implementation of a fully solvent-free process producing standard canola oil and new canola isolated proteins for food and feed applications. The primary product is

an isolated protein (~84% protein) high in well-balanced protein, appropriate for addition to food systems including nutritional applications, bakery and meat products. BioExx’s integrated plant is now able to process raw whole canola seed through true cold pressing and enzyme-assisted aqueous extraction, to produce food-grade proteins and oil without the use of any organic solvents, marking a major step forward in the oilseed processing field.

Appended to this document is the specification sheet of Advantexx™80, the first food-grade isolated Non-GMO canola protein produced from BioExx based on the new solvent-free process. It has excellent amino acid profile with an amino acid score greater than 1.05 indicating that it meets all the nutritional requirements for growing children over the age of 3 according to the FAO/WHO Recommended amino acid pattern (2007). With minimal heat treatment its functionality is similar to the native proteins. As shown in the figure 1 below, Advantexx™80 has high solubility across a wide range of pHs and compares well to other commercial concentrates made from Pea, Soy and Whey (test performed at 2% solution).

**Figure 1. Solubility Profile of Aqueous Processed Isolated Canola Protein as Compared With Other Proteins.**



While some previously investigated aqueous processes used enzymes to degrade the protein and enhance oil recovery, the new BioExx process retains the native integrity of the protein resulting in highly soluble and functional proteins. As the proteins are in their native state, further purification processes are possible to yield proteins of isolate quality (90%+ protein).

Canada is the world’s largest exporter of Canola and related seeds, with a total annual production of over 12,400,000 tonnes in 2009(Canolacouncil.org 2010). Canola and related seeds have been used as a food ingredient for at least two millennia. They are high in oil and protein and have a well-balanced amino acid profile. Recent mass-market acceptance of plant-based protein products has made recovery of proteins with balanced amino acid profiles much more lucrative than recovery of the oil. Currently, soy protein isolate values are in the range of US\$ 3.30 - US\$ 7.50/kg, while milk proteins are in the region of \$11-\$14/kg. Considering as well the value of the

oil (approximately US\$ 0.94-\$1.20/kg(canola council 2011) which has been the traditional driving force for oilseed cultivation, processes focused on protein recovery rather than maximum oil recovery are very attractive.

## **In the beginning there was only pressing**

As described in figure 2 below, the oldest and simplest method of processing oil containing seeds and fruits is by pressing out the oil in a manner similar to juicing a fruit. The first records of oilseed presses date back to 1100 BC and 500 BC in China and the middle east (Achaya 1994) and constituted the primary method of soy oil production prior to approximately 1920. Pressing is still the dominant process for the production of canola, rapeseed, brown mustard, sunflower and olive oils. In modern processing plants, pressing is normally performed in tandem with solvent extraction.

Continuous pressing uses an extruder to crush the seed against perforated walls to press out the oil. The intense pressure and heat created generally damages the seed protein. Prior to the press, the seed is normally flaked, and heated/conditioned to improve performance in the press, lower losses and improve the quality of the oil(Ward 1982). This treatment, however, can further degrade the meal with respect to the production of food grade proteins.

Modern press operations generally use high temperature oil pressing that denatures most of the seed protein. These high temperatures result in dark colour and very limited protein extractability. Modern processors use expanders to recover some of the extractability of the cake, which is otherwise very hard and compact. In less developed areas, small processors routinely produce oil by expression only (Head &Swetman 1999) producing a high oil meal generally useful only as animal feed (Head &Swetman 1999; Ward 1982).

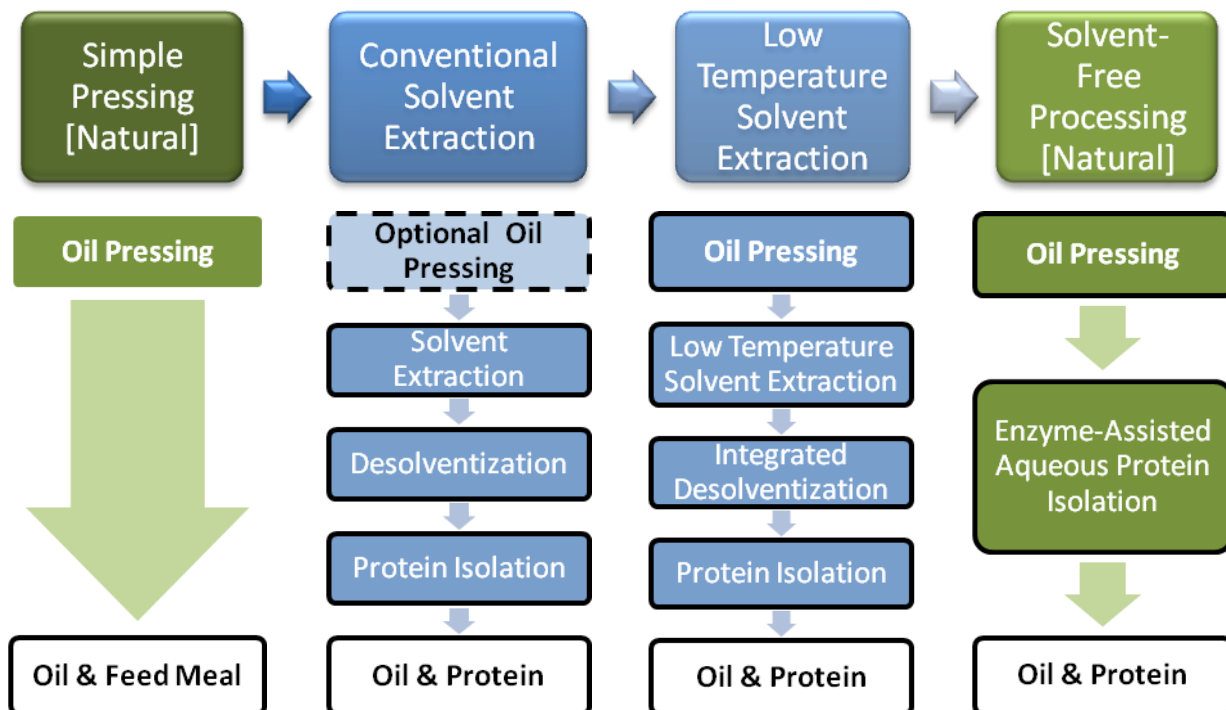
Cold press operations run at somewhat lower temperatures and pressures. However, mechanical action can result in quite high temperatures in the press if it is not actively cooled to run as a “true cold” press, which limits the damage to the proteins. The colder the pressing, the less the negative impact on the meal: oil removal is, however, impacted. Two-stage pressing can produce quite low oil meal of decent quality although it is still higher in oil than solvent processed meal.

Pressed oil from modern operations is usually further processed to improve the colour and flavour prior to retail sale. Traditionally the meal from pressing is used only for animal feed.

## Then came solvent extraction and hexane

The alternate method of obtaining oil is to dissolve it from the seed using a solvent, as is shown in figure 2. Because pressing can only recover a portion of the oil present in an oilseed, solvent extraction is commonly done on pre-pressed or partially de-fatted meal to complete the oil recovery. This is especially important in low oil content oilseeds such as soy. Nearly all soy processing facilities worldwide utilize solvent extraction. High oil-content seeds such as sunflower and canola/rapeseed are commonly pre-pressed down to 17%-20% prior to solvent extraction (Ward 1982) to enable use of the same solvent extraction equipment as soy, which contains approximately 19% oil.

**Figure 2. The Return to Natural Processing and the Development of Industrial Solvent-Free Protein Isolate Production**



The standard solvent in the oil extraction industry is industrial hexane, a petroleum distillate consisting of several related hydrocarbons but dominated by n-hexane. Hexane extraction allows recovery of virtually all the oil from seeds or pre-pressed meal through either a multi-stage bath in the solvent, or by trickle bed extractor. After oil extraction, the meal contains approximately 30-40% hexane. The meal must then be desolventized in order to maximize hexane recovery and, for health reasons, eliminate traces of the solvent. In conventional processing, high temperatures and steam are used to remove the solvent. The meal is generally toasted at the same time to aid in digestibility, with temperatures regularly exceeding 100°C, well above the temperatures for

irreversible damage to the protein. This significantly limits the extractability and functionality of the proteins (Marsman et al. 1997). Enzymatic treatment of the meal can improve solubility and functionality (Marsman et al. 1997).

Soy processed for production of food proteins uses a different desolventizing process to produce “white flake” with a much higher extractability and high nitrogen solubility. Through a hexane flash and vacuum loop system, the white flake process removes the hexane without subjecting the meal to high temperature. Unfortunately this process, is considerably less efficient at hexane recovery than conventional toaster desolventizing resulting in higher losses. White flake is used for the production of both protein concentrates and protein isolates.

Hexane is listed as a Hazardous Air Pollutant under the 1990 amendments to the U.S. Clean Air Act and as such, the U.S. FDA is considering delisting it as a safe solvent (Wan et al. 1995). While still acceptable under the Codex Alimentarius, its status is tenuous. An industrial alternative is necessary as its delisting seems inevitable. Current EPA guidelines limit the release of HAPs for new oilseed processing plants to 0.2 gal/ton seed (soy) processed. This however applies specifically to traditional large scale soy processing plants which incorporate desolventizing/toasting to the meal. White flake processed meal use, which is termed a specialized recovery system and the EPA guidelines, limit hexane losses for these plants to 1.5 gal/ton seed produced. Since protein isolates require approx. 3 ton seed to produce 1 ton of finished isolate, up to ~4 gallons of hexane can be released per ton of soy protein isolate produced. For canola processing, estimated industrial hexane losses can range up to 2.0 l/ton (Dahlén & Lindh 1983) between soy and Canola, hexane losses to the environment are quite substantial.

## **The quest for alternate solvents and processes**

The listing of hexane as a hazardous air pollutant has generated great interest in non-HAP solvents and alternative solvent systems. Leading the list of alternative non-HAP solvents are the related compounds of Heptane, Isohexane(2-methyl pentane), and cyclohexane. These are all components of industrial hexane, and have excellent oil solvent characteristics, recovery costs similar to hexane, and run at temperatures within 25°C of hexane. While their toxicity seems lower than that of n-hexane, all are very flammable and pose health risks when used in plant, and face the same issues of solvent carryover and need for desolventization. Indeed since the boiling point of Heptane is approximately 30°C higher than that of hexane, desolventization of the meal would require more energy and higher temperatures, leading to greater damage to the meal.

Solvents such as acetone, ethyl and isopropyl alcohol also show great promise and have generated much industry interest as green alternatives. However, most would require extensive redesign and face substantial obstacles. While ethanol is inexpensive, easy to biologically treat, and may be regarded as nontoxic, its solvent capacity for oil is limited, especially as water is introduced (40% oil in 100% EtOH @ 70 °C, <5% oil in 95% EtOH @ 78 °C). Being a good solvent for many undesirable seed materials such as phospholipids and phenolics, it is useful for cleanup of oil, but poor as an extractant. Isopropyl alcohol (IPA) is more promising in many ways (Lusas 1997, Baker & Sullivan 1983, Shah & Venkatesan 1989) as it is inexpensive and acceptable to the Codex Alimentarius although not for processing of infant foods. While somewhat more toxic than ethanol, it is much better at dissolving oil especially when using pure IPA. The presence of

water lowers the solvent capacity somewhat (Baker & Sullivan 1983; Lusas 1997). Unfortunately recovery requires 50%-90% more energy than for hexane and worsens with the addition of water (Lusas 1997). In addition, regeneration of pure IPA is extremely expensive due to the presence of an azeotrope at 13% water/87% IPA. From an environmental viewpoint, isopropyl alcohol is less noxious than hydrocarbon alternates. Unfortunately, such a process would still pose toxicity and fire hazards and the meal would still require some desolventization prior to any further processing.

## **A new process eliminates solvents**

BioExx was founded on the premise that extraction with minimal thermal treatment to the seed, will result in substantially higher value in protein isolates by retaining the native solubility and functionality, while enhancing protein recovery. BioExx protein isolation was previously built on the combination of true cold pressing of the seed followed by extraction of the residual oil using low-boiling point solvents as shown in figure 2. This process allowed complete desolventization at temperatures well below the denaturation points of the proteins and produced highly functional proteins with high solubility. Solvent was fully recovered through an integrated process, leaving no trace of solvent in resulting meals or products.

Unlike most producers, where the value of the oil is the first consideration in the process, producers such as BioExx focus on the value of the protein allowing them to implement process changes that would not be possible with other producers. Environmental responsibility and sustainability has now led to the development to a new generation of processes that completely eliminates solvents from the process while retaining many of the key aspects of the previous generation.

## **Aqueous extraction can now produce low-fat, food-grade protein from Canola seed without the use of hydrocarbon solvents**

Production of high-purity protein isolates free of anti-nutritional compounds through a solvent free, fully-aqueous process offers significant advantages. Previous processes relied upon complete de-fatting of the seed through solvent extraction prior to protein extraction and purifications. Unfortunately, hexane used during de-fatting poses specific safety concerns due to its flammability, volatility, neurotoxicity and hepatotoxicity, resulting in its recent designation as a harmful air pollutant. Hexane use requires stringent design, significant capital cost for solvent recovery, and expensive operator training. Hexane has been shown to remain associated to the spent meal of soy and other oilseeds. Hexane loss and release can be substantial during traditional protein production.

Since aqueous processing is ultimately required for the isolation and purification of protein from seed or meal, a fully aqueous process was a natural fit. As shown in figure 3, the development of the solvent-free process resolves many of the issues plaguing modern isolate production.

**Figure 3. Development of Oil and Protein Technologies**

<b>Pressing</b>	<b>Solvent Extraction</b>	<b>Modified Solvent Extraction</b>	<b>Cold Pressing &amp; Aqueous Extraction</b>
<b>Ancient</b> Since 1100 BC	<b>Turn of the 20<sup>th</sup> century</b> Since 1870	<b>Turn of the 21<sup>st</sup> century</b>	<b>Modern</b>
Natural	Solvent based Hazardous Air Pollutants: First: Benzene Modern: Industrial Hexane	Solvent based Low HAP alternatives Some low temp options	Natural Water based
Organic compatible	Forbidden	Forbidden?	Organic compatible
Historically inefficient Improved modern efficiency	Efficient Oil recovery	Generally Efficient Oil recovery	Efficient Oil and Protein recovery
Damage to protein value; emphasis on oil with meal and protein considered of low value	Generally: Damage to protein value and functionality Specialized: protein value retained (soy white flake)	Generally Damage to protein value and functionality Specialized: protein value retained (low temperature solvent extraction)	Protein Value and functionality retained
Safe operation	Special containment and recovery required Fire and explosion hazards Operator inhalation hazard	Special containment and recovery required Fire and explosion hazards Operator inhalation hazard	Safe operation
No solvent release	Environmental release of solvent up to 1.5 gal/ton seed	Potential Environmental release of solvent	No solvent release
No solvent residues	Potential solvent residues in oil and proteins	Potential solvent residues in oil and proteins	No solvent residues

Solvent free aqueous processing from non-GMO canola/rapeseed seed is new and viable:

- i. It produces oil and protein on commercial scale
- ii. It results in proteins competitive to whey and soy;
- iii. It uses no organic solvents allowing retention of natural status and allows applicability into organic market; and
- iv. It is economically competitive with other vegetable oils and proteins.

Currently, there is no other industrial process that meets all of the above demands.

Aqueous extraction uses economical, plentiful, natural water, rather than hydrocarbon solvents with their intrinsic fire, explosion and health hazards. This leads to a better environmental footprint while improving the overall economics. As lifecycle modeling of the impact of industrial processes becomes more prevalent, minimal-polluting processing will grow in importance. This new process is helping lead the way, and BioExx has pioneered the technology.

For further information on BioExx Proteins please contact:

**David Balke**  
**Food Application Specialist**  
BioExx Specialty Proteins, Ltd.  
[dbalke@bioexx.com](mailto:dbalke@bioexx.com)  
416-588-4442 x118

## Product Description

The BioExx isolated canola protein **Advantexx™ 80** is made from non-GMO Juncea-variety Canola seed through the combination of true cold oil pressing and our proprietary enzyme-assisted fully aqueous protein isolation technology. Advantexx™ 80 may be used in either human food products including:

- >> Human food products :
  - > Bakery products
  - > Meat products
  - > Vegetarian food products and meat analogues
  - > Nutritional and high protein bars, drinks and supplements

## Technical Data

Advantexx™80 canola protein is of high nutritional quality and capable of providing adequate amounts of all essential amino acids.

- >> Complete protein – PDCAAS ≥ 1.0 (ref. FAO 2007)
- >> Uniquely high in both lysine and sulphur containing amino acids compared to other vegetable proteins.
- >> >40% higher in the sulfur containing amino acids (methionine + cysteine) than typical soy protein and 75% higher than typical pea protein.
- >> Rich in the muscle building branched-chain essential amino acids (valine, leucine, and isoleucine)
- >> Free of phytoestrogens.
- >> Non-GMO, natural product.

## Functional Properties

Advantexx™80 canola protein has unique functional properties including:

- >> Excellent water solubility as compared to soy and pea proteins.
- >> Excellent emulsifying and foaming properties as compared to whey proteins and egg yolk.
- >> Performs particularly well in the applications requiring emulsifying and foaming.
- >> Excellent gel forming property and gel firmness.

**ADVANTEXX™80 Typical Analysis**

Physical Description		As Is	
Appearance	Spray Dried Tan Powder		
Flavour	Mild		
pH	7 ± 0.5		
Solubility	>60% @1g/100ml pH7		
Proximate Analysis		Minimum	Typical
Protein (Leco nx6.25)	>73% as is >80% dwb	80% as is 83% dwb	
Moisture	<7%	5%	
Ash	<4%	3.2%	
Fat (AOCS Ba 38)	<2%	<2%	
Crude Fiber	<0.5%	<0.1%	
Microbiological Profile			
Standard Plate Count [cfu/g]	<10,000	<1,000	
E.coli	Negative	Negative	
Salmonella	Negative	Negative	
Nutritional Data			
Saturated Fat	0.0%		
Cholesterol	0.0%		
Trace Components		Minimum	Typical
Total Glucosinolates	<1 µmol/g	<0.2 µmol/g	

Typical Amino Acids	(G/100G Protein)
Alanine	4.6
Arginine	7.3
Aspartic Acid	10
Cysteine	1.8
Glutamic Acid	18.8
Glycine	5.5
Histidine <sup>†</sup>	3.1
Isoleucine <sup>†</sup>	4.0
Leucine <sup>†</sup>	7.6
Lysine <sup>†</sup>	5.1
Methionine <sup>†</sup>	2.1
Phenylalanine <sup>†</sup>	4.4
Proline	5.6
Serine	5.4
Threonine <sup>†</sup>	5.0
Tryptophan <sup>†</sup>	1.5
Tyrosine	3.2
Valine <sup>†</sup>	5.0

Sulfur amino acids

<sup>†</sup> essential amino acids.

**Granulation**

Min 50% through #80 mesh U.S. Standard Screen

**Packaging**

Available in 20 kg (44 lb.) net weight, multi-wall, poly-lined paper bags. Custom packaging available.

**Storage**

Recommend shelf life is 12 months from date of manufacture if stored below 25°C (75°F) and 60% relative humidity