

Scalable Extraction and Purification Processes for Nutraceuticals and Health Supplements



Sandeep B. Kale, PhD (Tech)

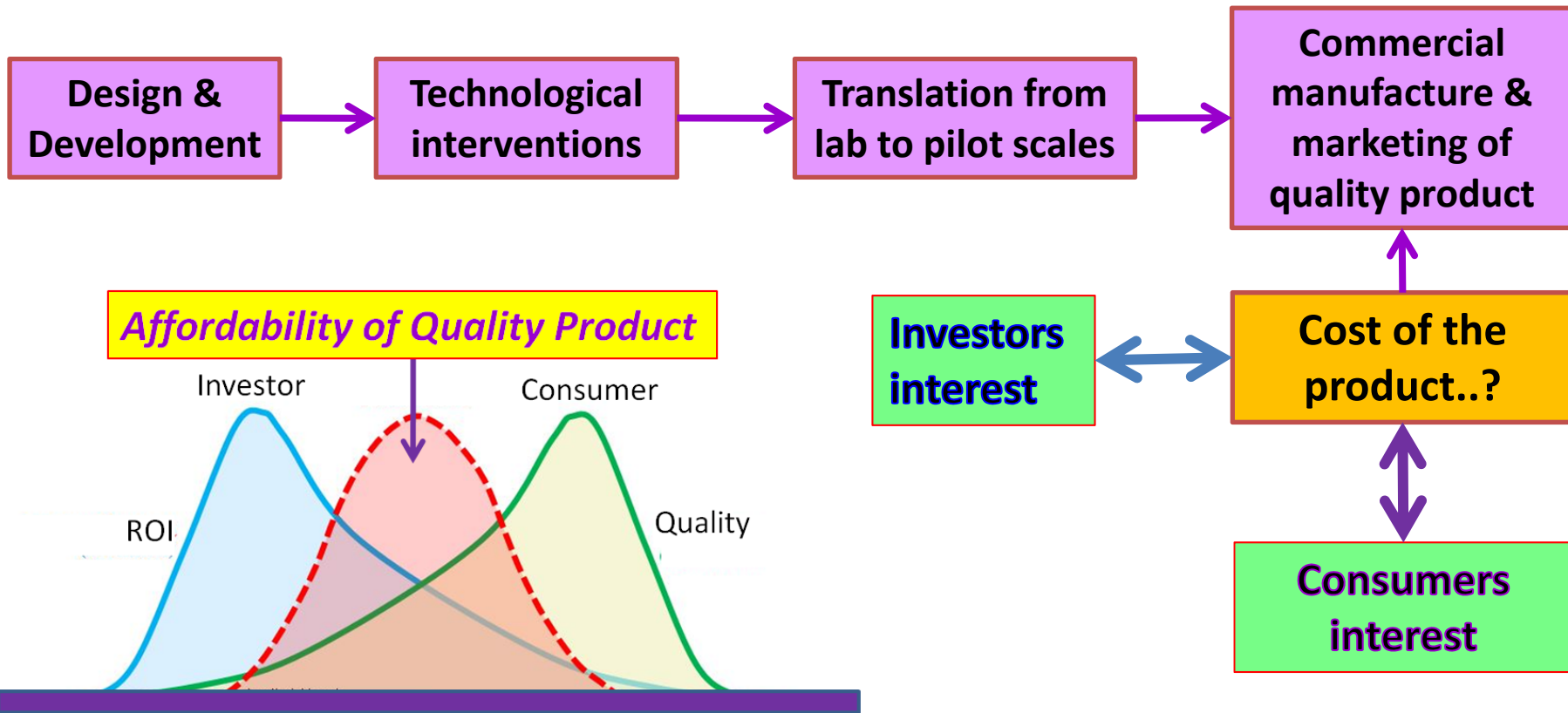
QbD Purple Advanced Technologies Pvt. Ltd. (QPAT)
International Biotech Park, Hinjewadi, MIDC, Pune-57
sanykale@gmail.com

PFNDA Seminar, 5th October 2018

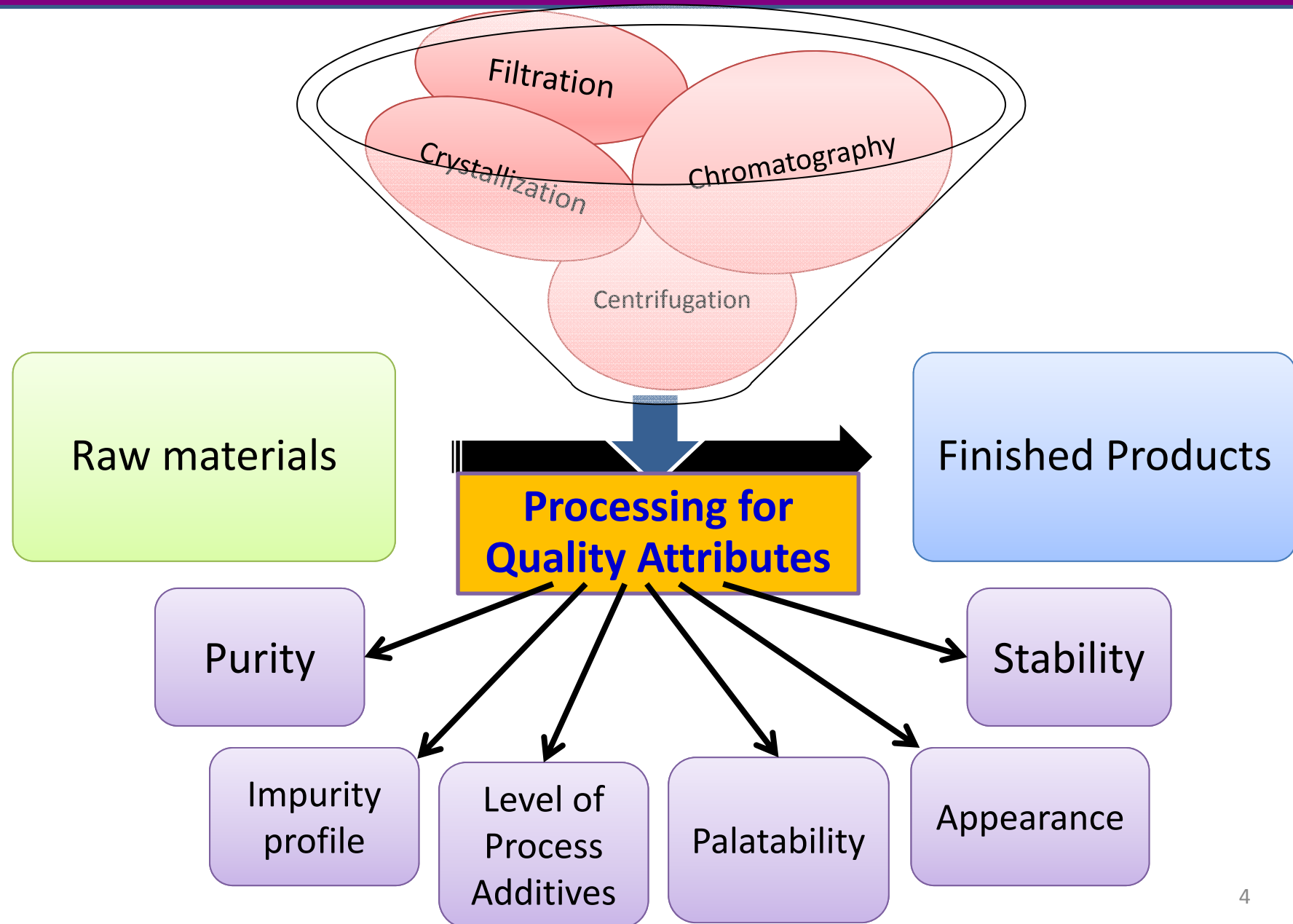
Research areas

- Natural products including phytopharmaceuticals and nutraceuticals
- Biocatalysis and fermentation
- Product and process characterization
- Secondary agriculture (value addition to Agri-produce)
- Improved feed and food ingredient
- Waste to wealth
- Biopharma and Biologicals including, monoclonal antibodies plasma proteins, urinary hormones, peptides etc.
- **Scale up of processes from lab to pilot and commercial scale**

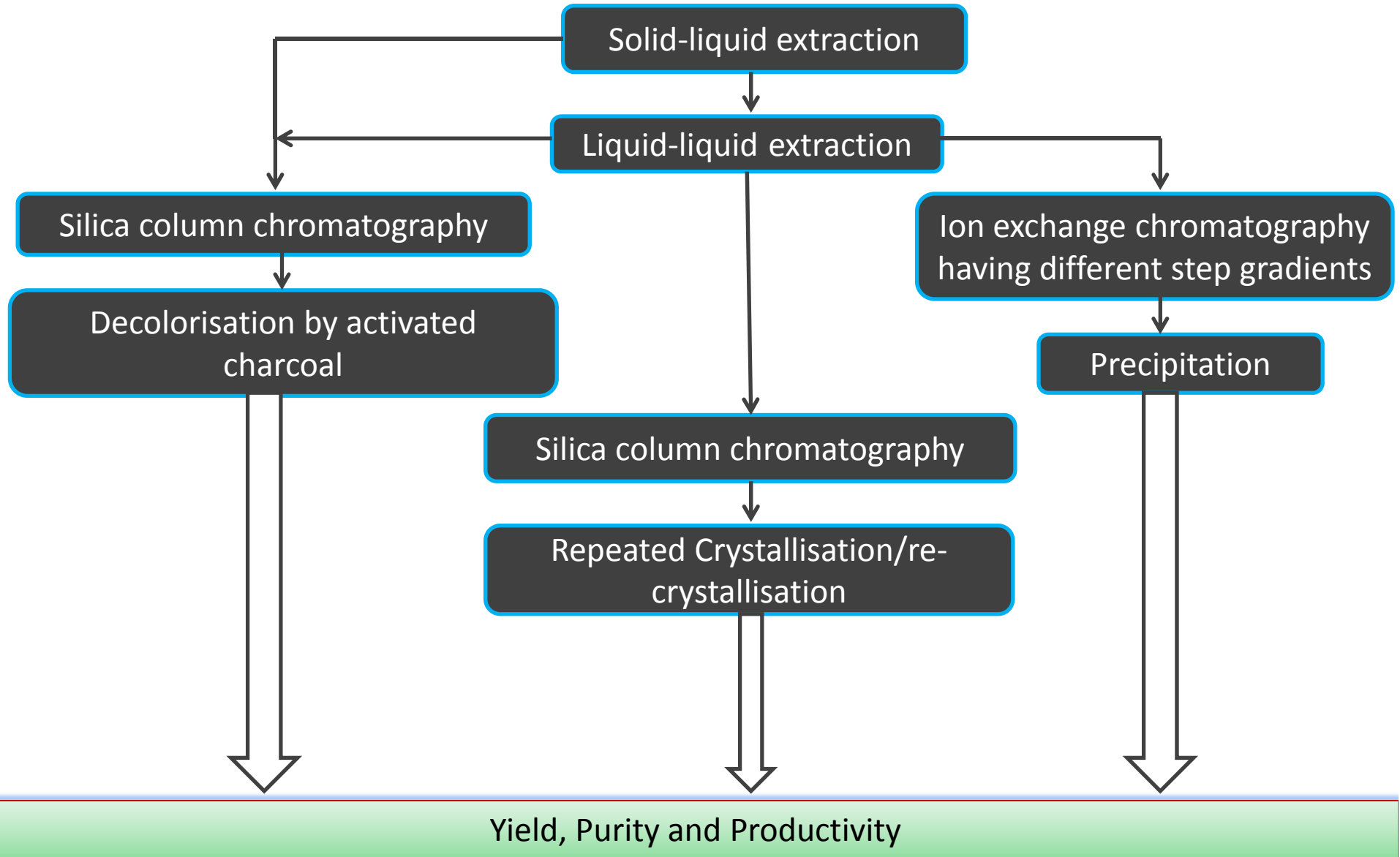
Affordability-The GAP



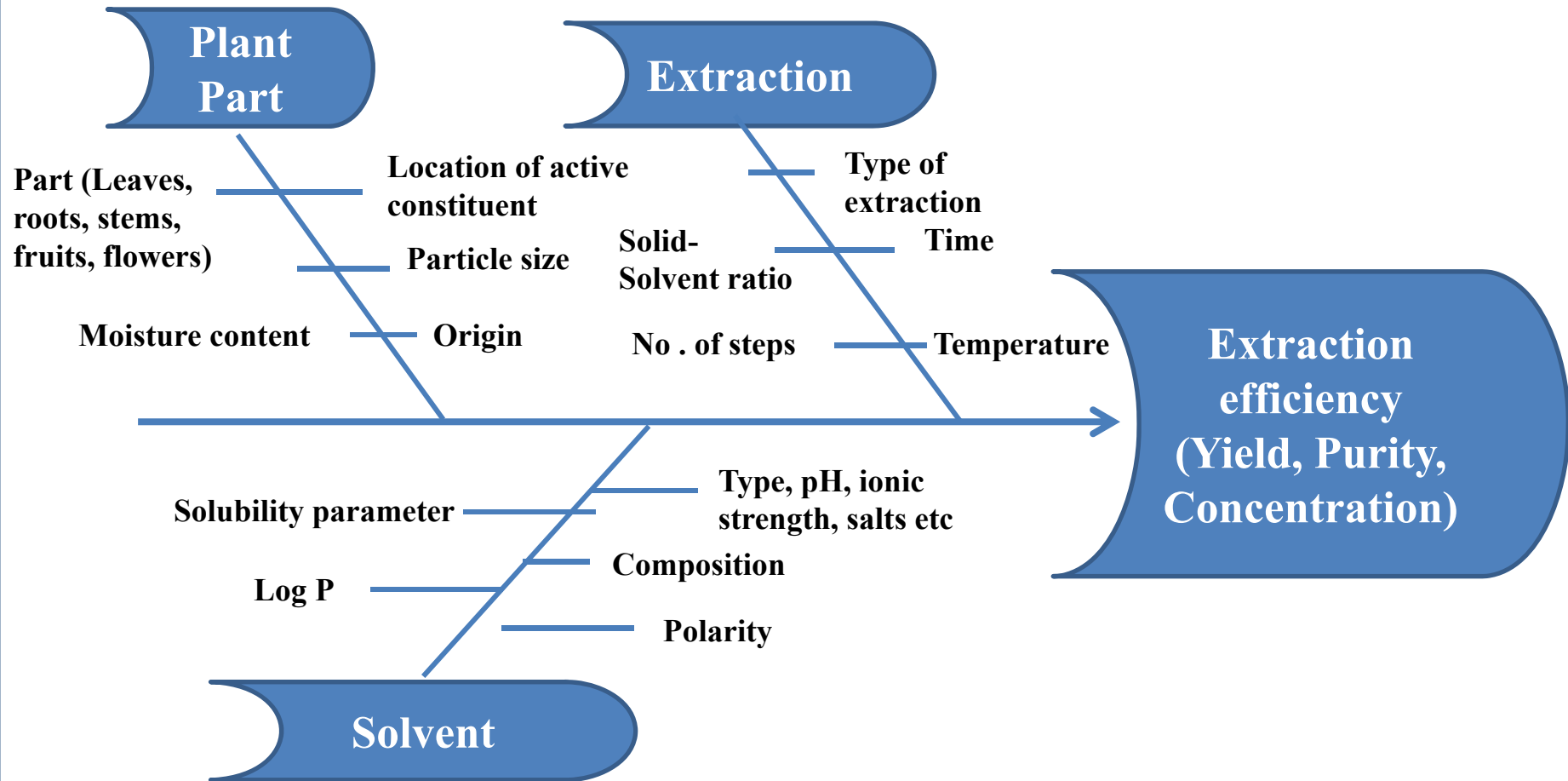
Bioprocessing for quality attributes



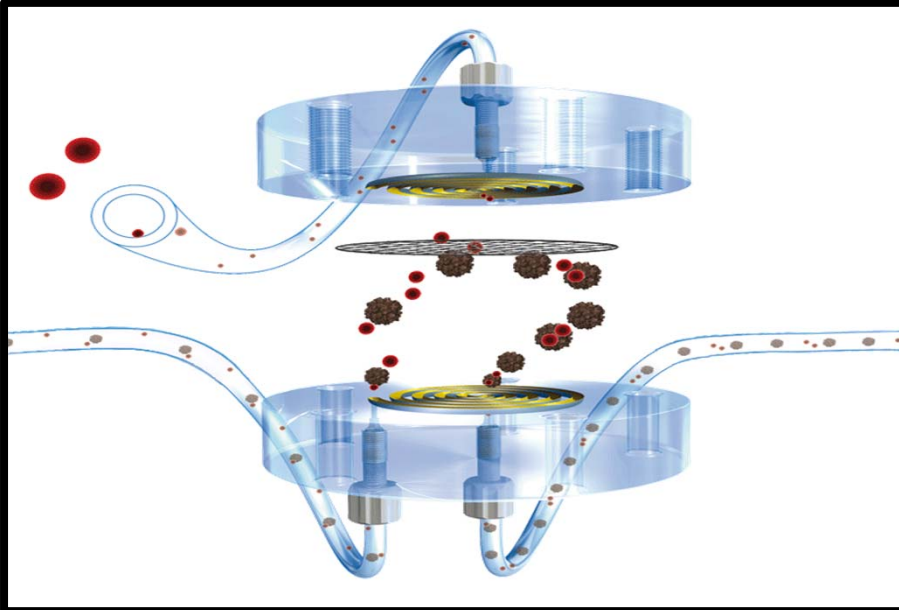
Process Scale Extraction and Purification



Extraction

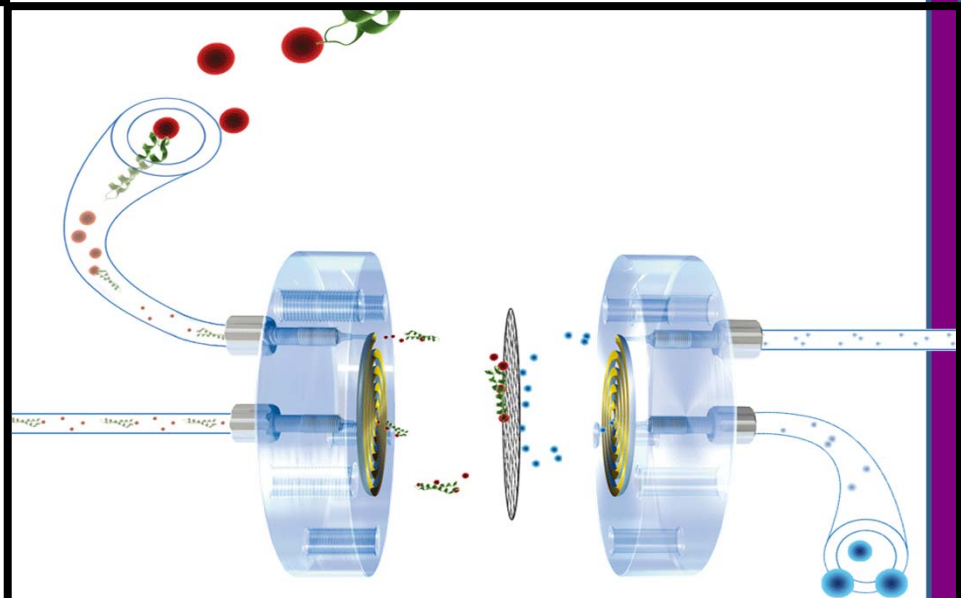


Membrane filtration: MF/UF/NF/DF/RO



- Isolation and fractionation of biopolymers (proteins)
- Removal of proteins or polymeric mass from small bioproducts
- Desalting and buffer exchange
- Concentration of biopolymers or small mol. Wt. bioproducts

- Recovery of enzymes during biotransformation, for recycle
- Integration with adsorptive separation

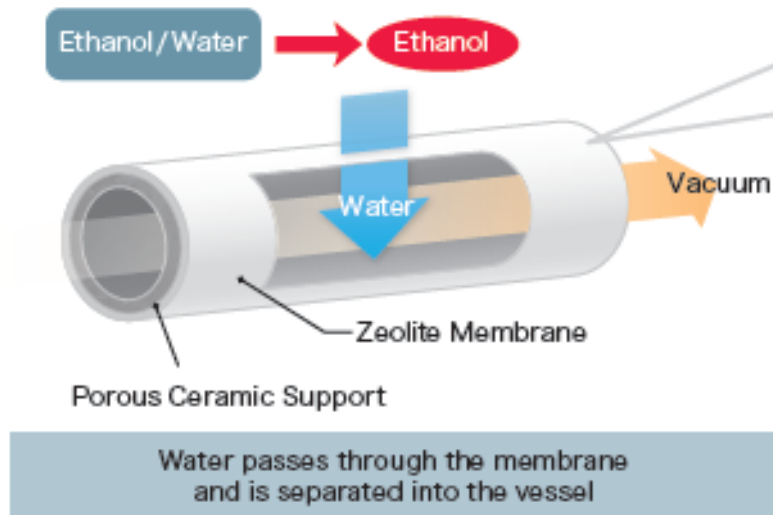


Application of Membrane for Food Dehydration

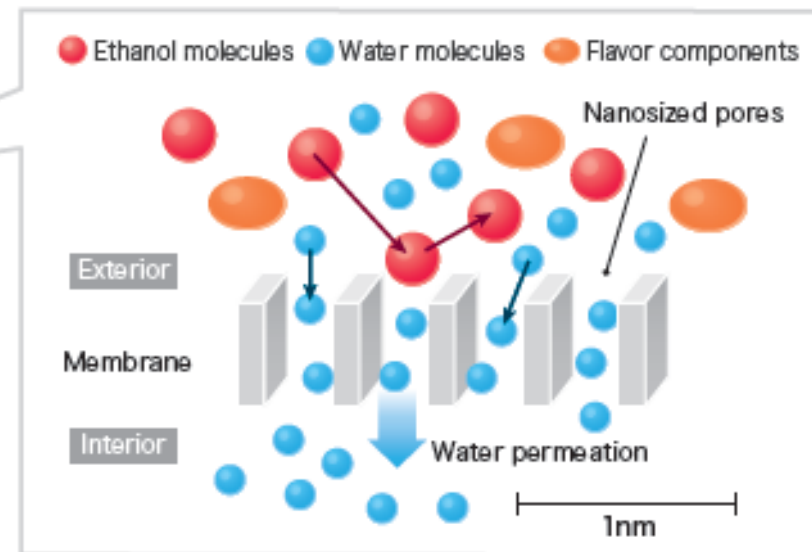
Membrane for food dehydration and concentration can be used with a variety of liquid foods, making it possible to safely concentrate and dehydrate without the use of heat while preserving flavor and fragrance components.

It's now possible to selectively dehydrate and concentrate a variety of foods without heat.

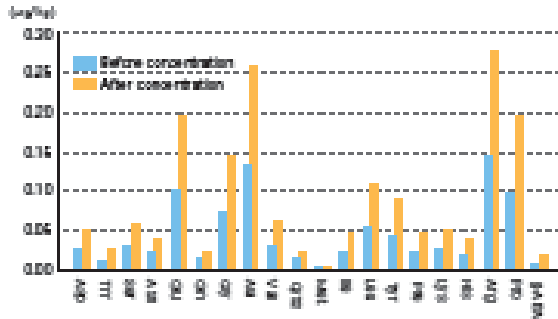
■ Example of concentration of ethanol/water solution



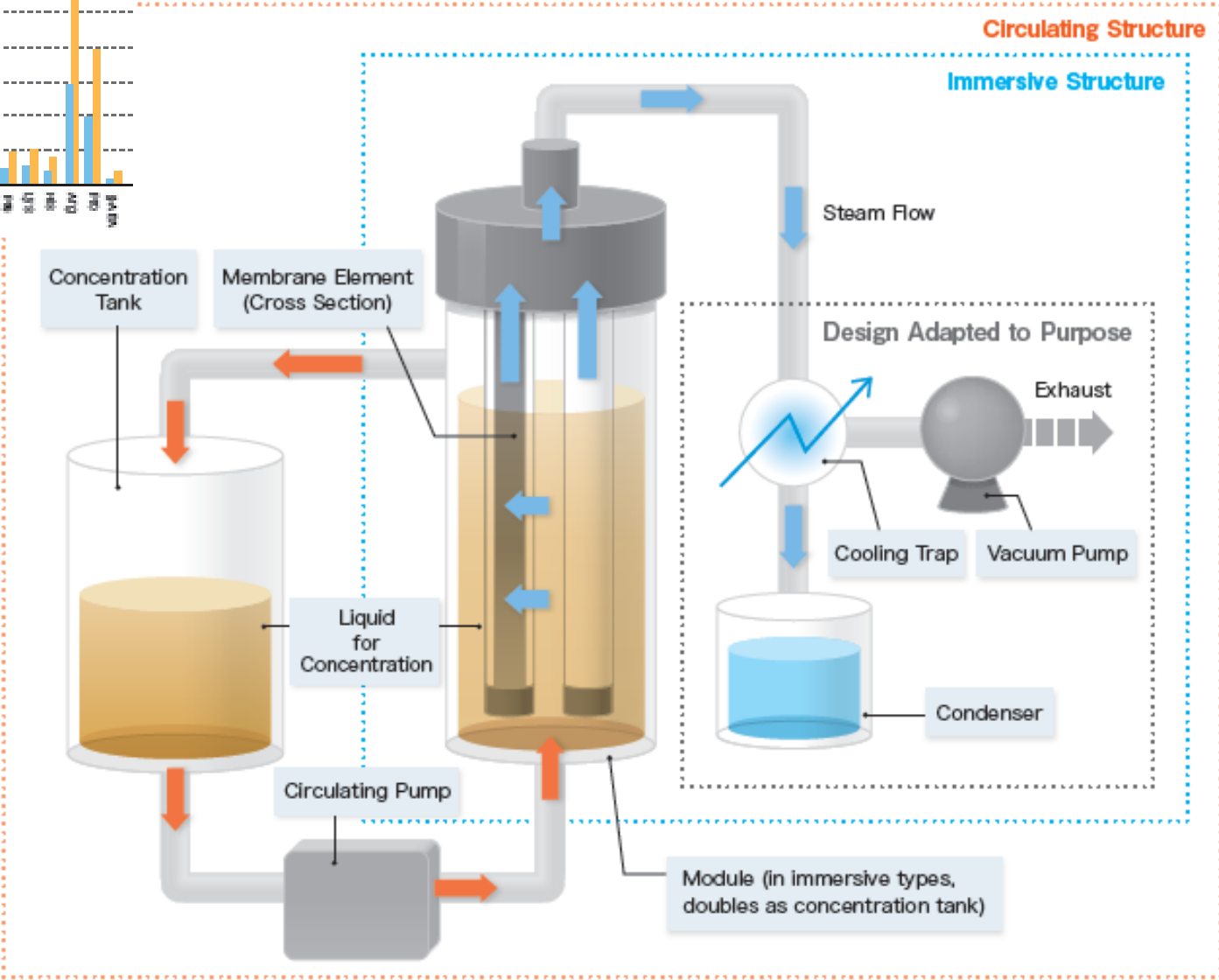
■ Cutaway Schematic of Membrane and Concentration Concept



Application of Membrane for Food Dehydration

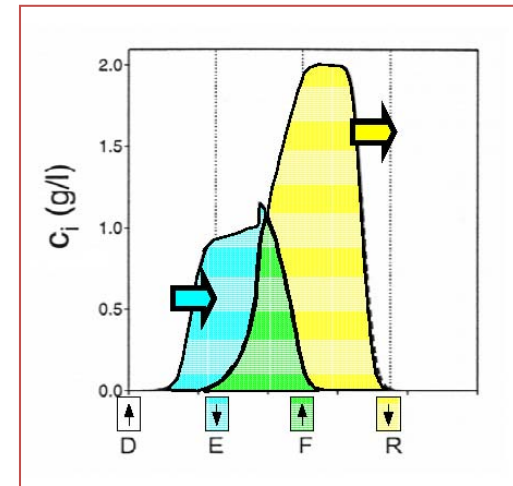


- a.Reduced cost
- b.Small footprint
- c.Improved productivity
- d.Zero emission

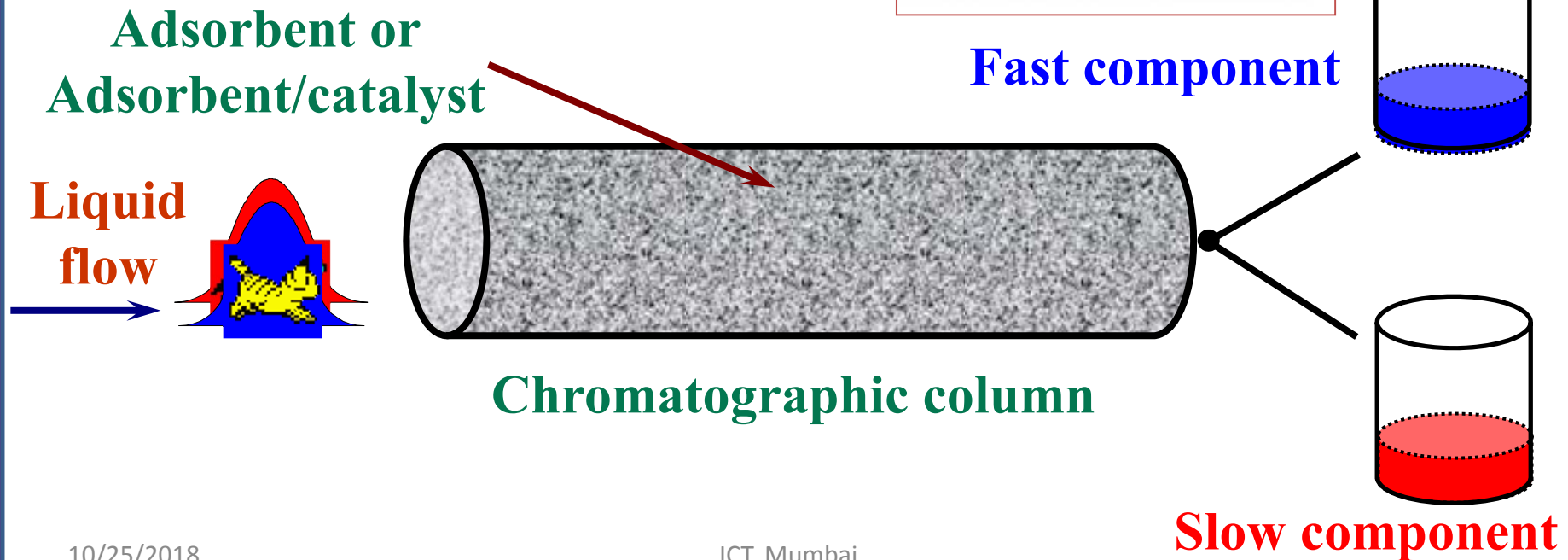


Adsorptive Chromatography

- Separation is achieved by preferential adsorption of components in a chromatographic bed.
- Selective adsorption leads to different migration velocities



Isocratic
and/or
Gradient

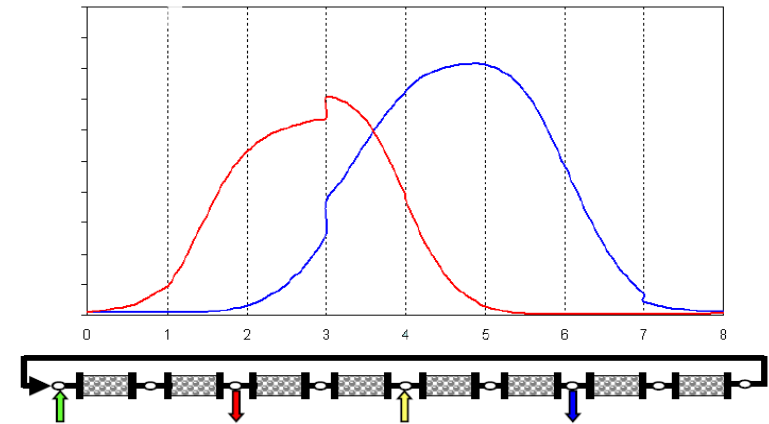
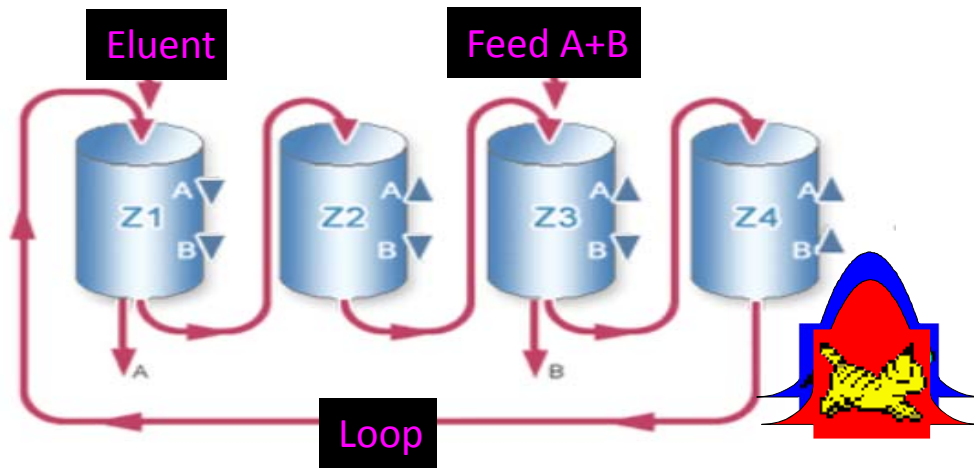


10/25/2018

ICT, Mumbai

Chem. Eng. Sci., 49, p469, 1994

Chromatography – ISMB

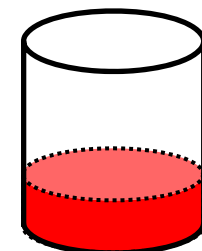
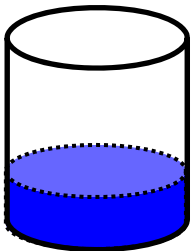


Liquid flow

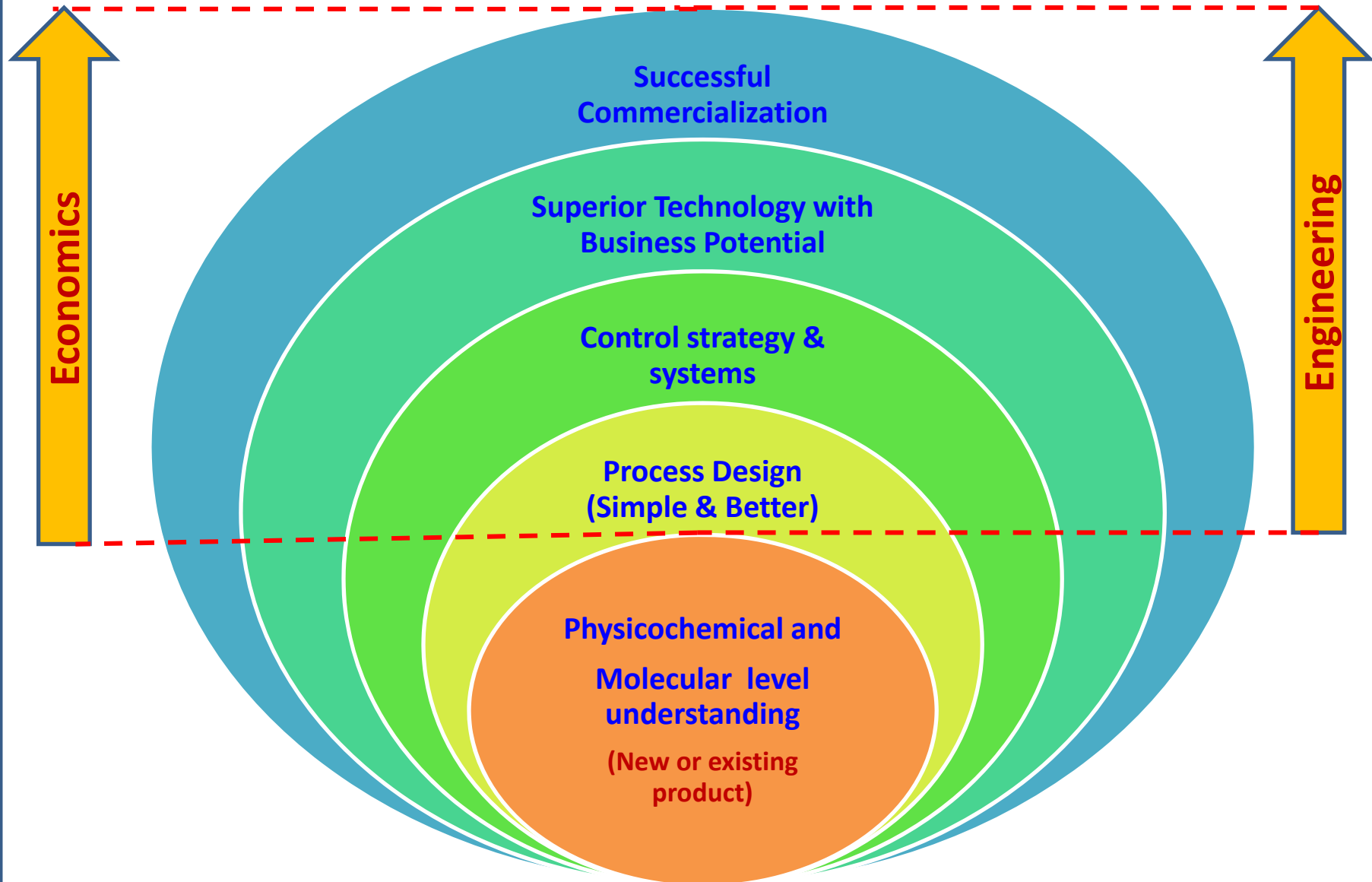
$$\sigma_T > 1$$



**Intermediate solid Flow:
Countercurrent flow**



Process Synthesis



Why Bioactive Compounds ??

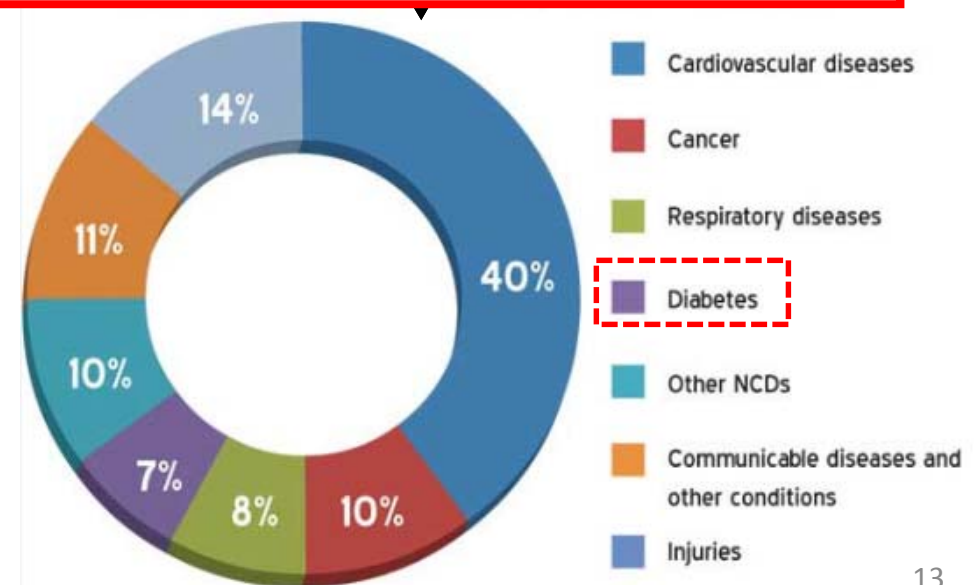
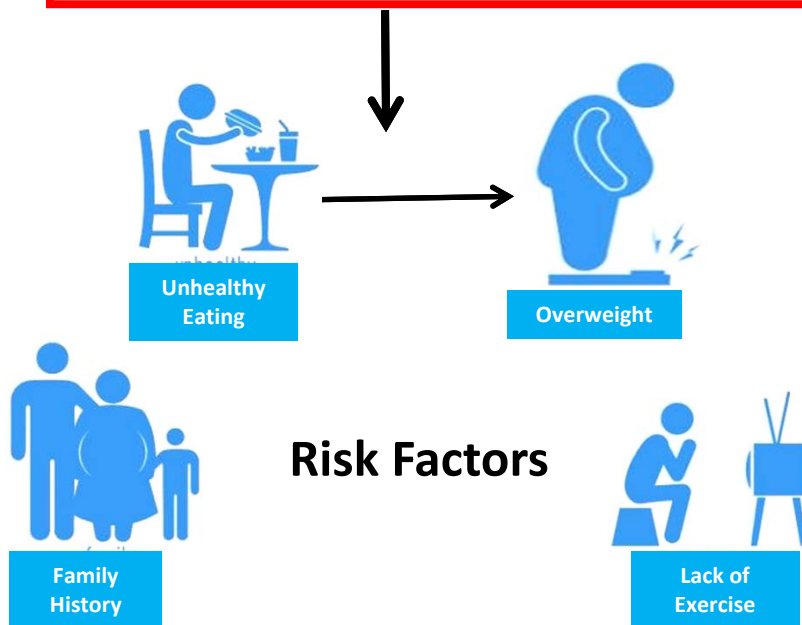
“Food is Medicine”

- NCDs
- COMMUNICABLE DISEASES, MATERNAL, NEONATAL, AND NUTRITIONAL DISORDERS
- INJURIES

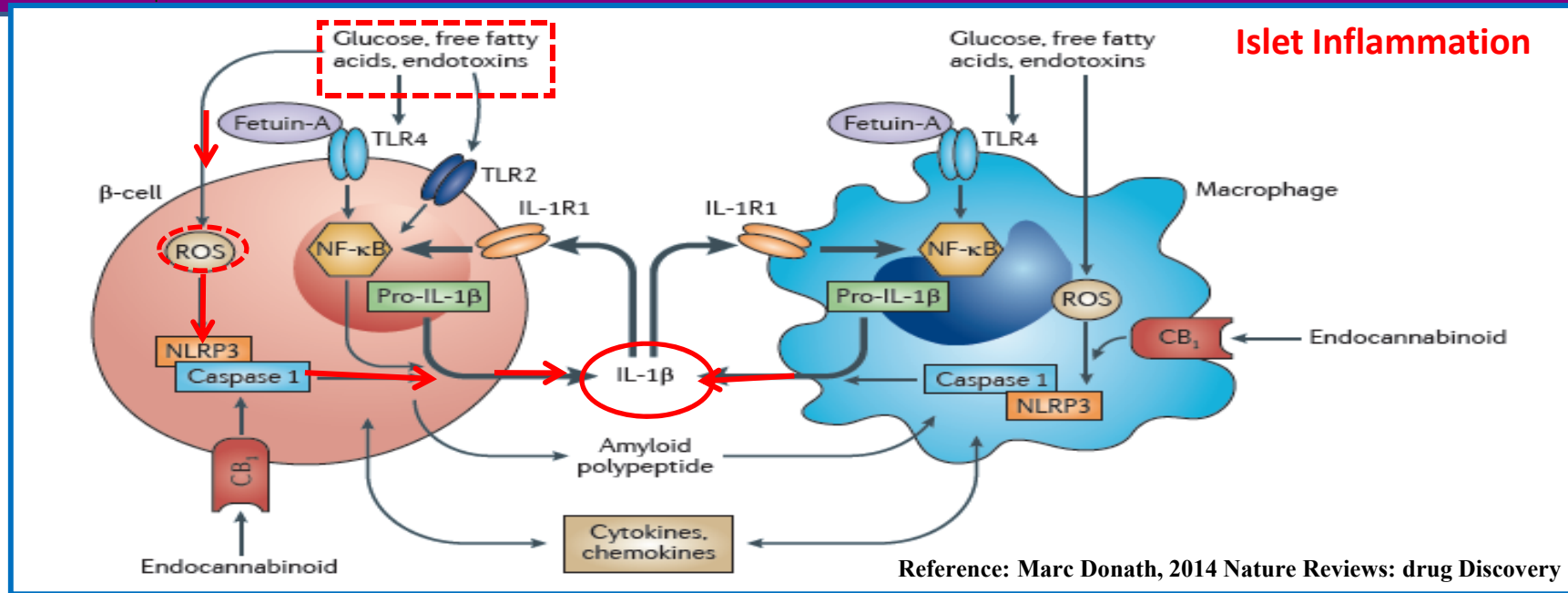
✓ Functional Food

“Food which is demonstrated to positively affect one or more physiological functions, so that it is able **to increase the well-being and/or to reduce the risk to suffer from a disease**”

✓ **Bioactive Compounds** are **extra-nutritional** functional ingredients responsible for the **functional bioactivities** like antihypertensive activity, **hypoglycemic**, hypocholesterolemic effect, antioxidant properties, probiotic or prebiotic effects, or regulatory effects.



Unmet Need



Need for New Bioactive???

Current solutions

Natural and allopathic medicines



Potent, Safe, Nutritious and Low cost bioactives for management of blood glucose as well as associated inflammation



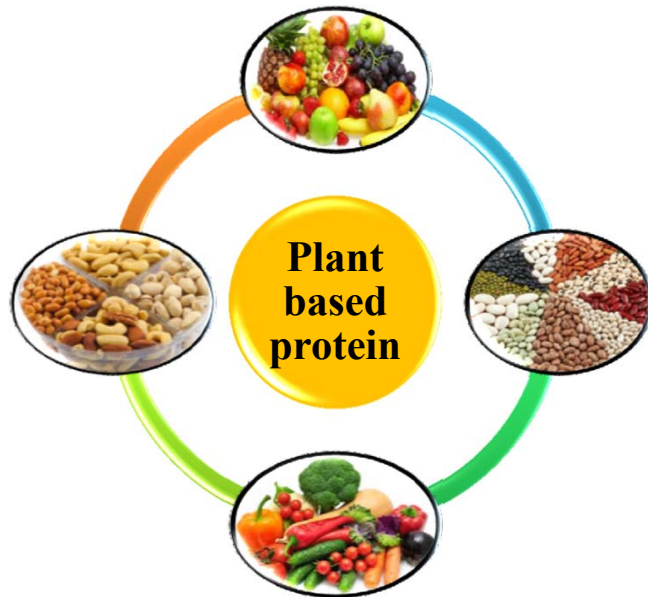
Exercise



Insulin injection

Solution	Cost for 10 pills	Daily dose frequency	Remark
Glycomet GP (USV) (synthetic)	39	Twice	Causes vitamin deficiency
Diabohills (mixture of herbal extracts)	41	Twice	Non nutritional

Plant Proteins



Seed Storage Proteins

Albumins

Water Soluble

Globulins

Saline soluble

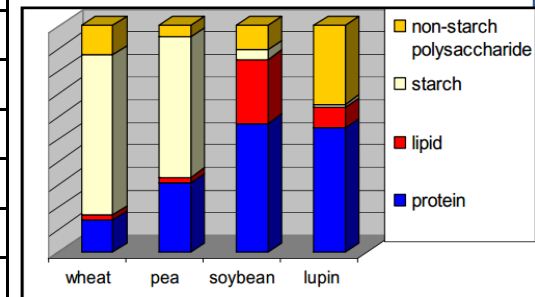
Prolamins

Alcohol soluble

Glutelins

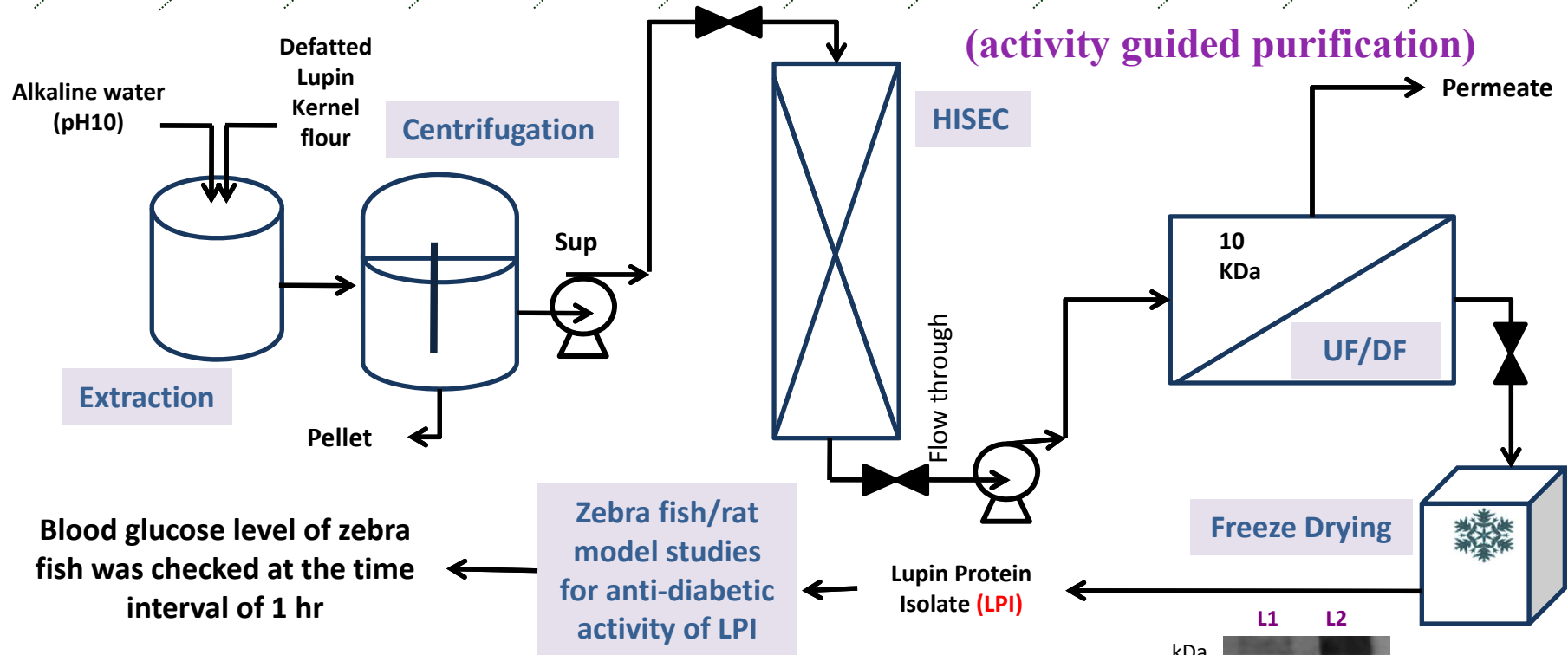
Acid/alkali soluble

Plant Source	Protein (%)	Albumins (%)	Globulins (%)	Prolamins (%)	Glutelins (%)
Wheat	10.6	6-10	5-8	35-40	40
Rice	7	2-6	12	4	80
Barley	11	3-5	10-20	35-45	35-45
Maize	9.8	4	4	60	26
Sorghum	8.3	2-7	2-10	35-60	20-35
Soybean	40-50	--	90	--	--
Pea	23-28	15-25	50-60	--	--
Chickpea	19-20	8-12	53-60	3-7	19-25
Lupin	35-40	25	75	--	--
Canola/Rapeseed	40	20	60	2-5	15-20



Reference: Lupins for Food & Health
-Dr Mark Sweetingham

Activity guided purification

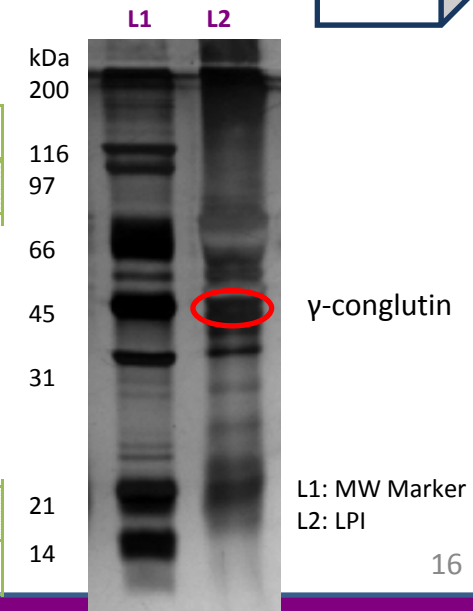


Fractions	Wt. of solids (gm)	Amount of protein (gm)	% Protein
Defatted flour	7.08	3.50	49.44

Results :

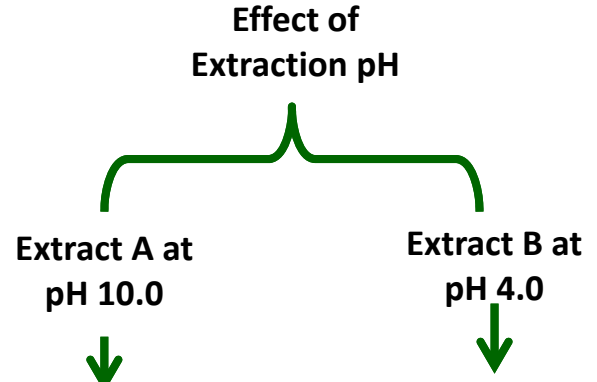
- Lupin protein Extracted : **91.4%**
- Assay purity of lyophilized protein isolate: **89.46%** (total protein)
- Overall Yield: **28%**

Diafiltered	1.30	1.06	81.49
Lyophilized protein isolate	1.09	0.98	89.46



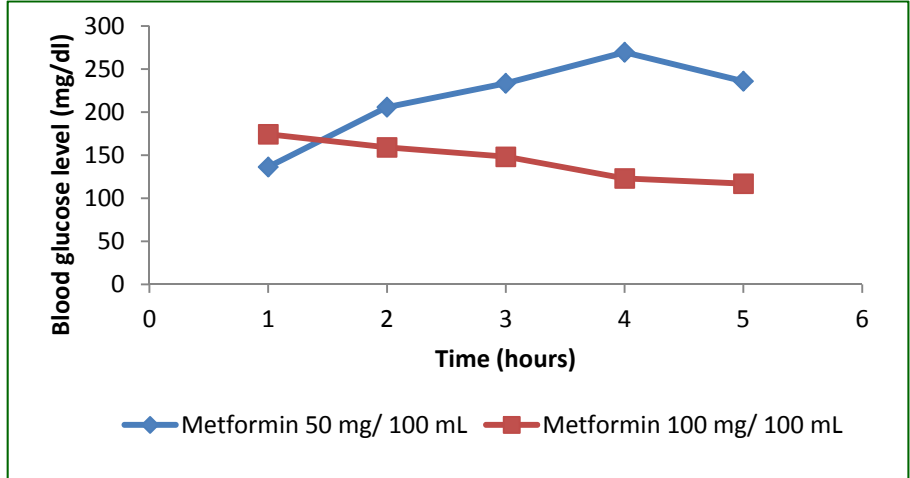
Anti-diabetic activity study

(Activity guided purification & comparison with Metformin)

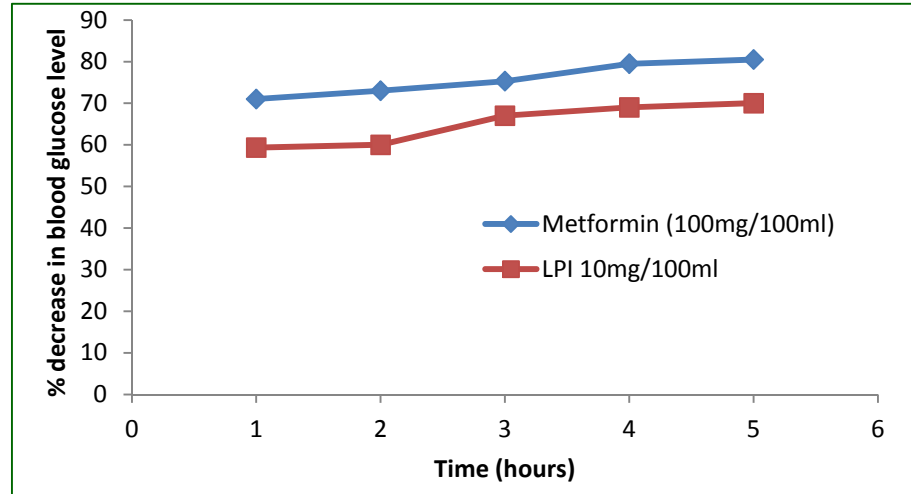
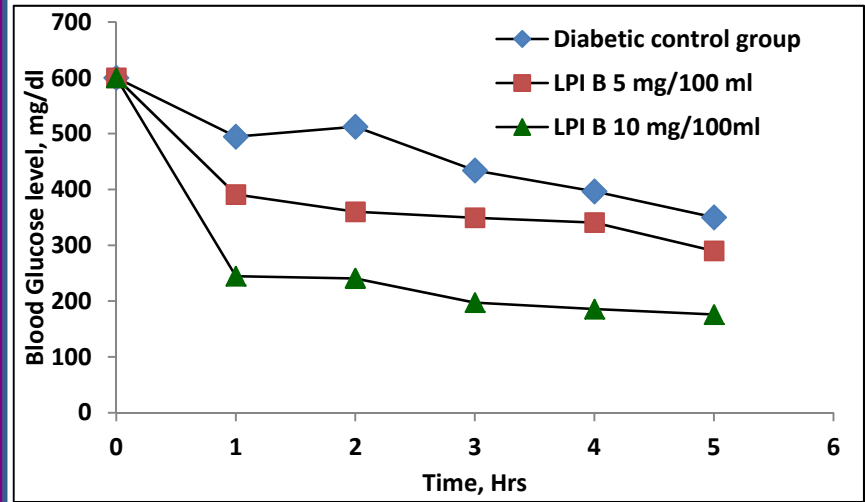


50% reduction in blood glucose in 4 hrs

Fast reduction of blood glucose level was observed (High potency...?)



Anti-diabetic Activity at two different dosages of Metformin

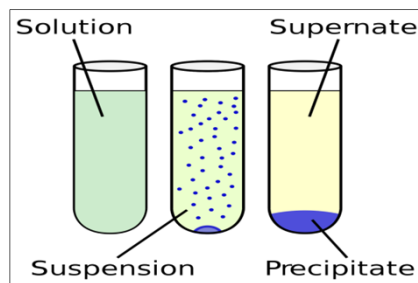


Type B extract was found to be 10 times more potent than Metformin

Process Designing

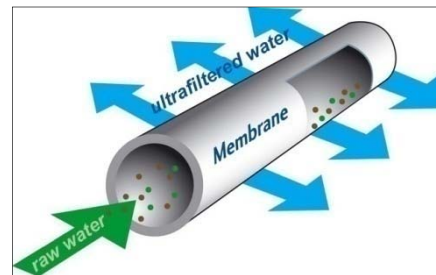
Precipitation

- **Isoelectric point based precipitation**
- **Divalent metal ion based interaction**



Membrane Process

- **Microfiltration**
- **Ultrafiltration**
- **Diafiltration**

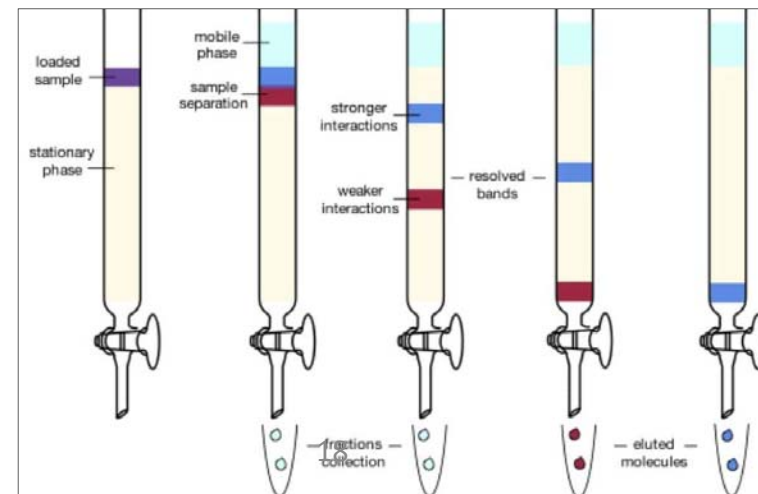


Chromatography

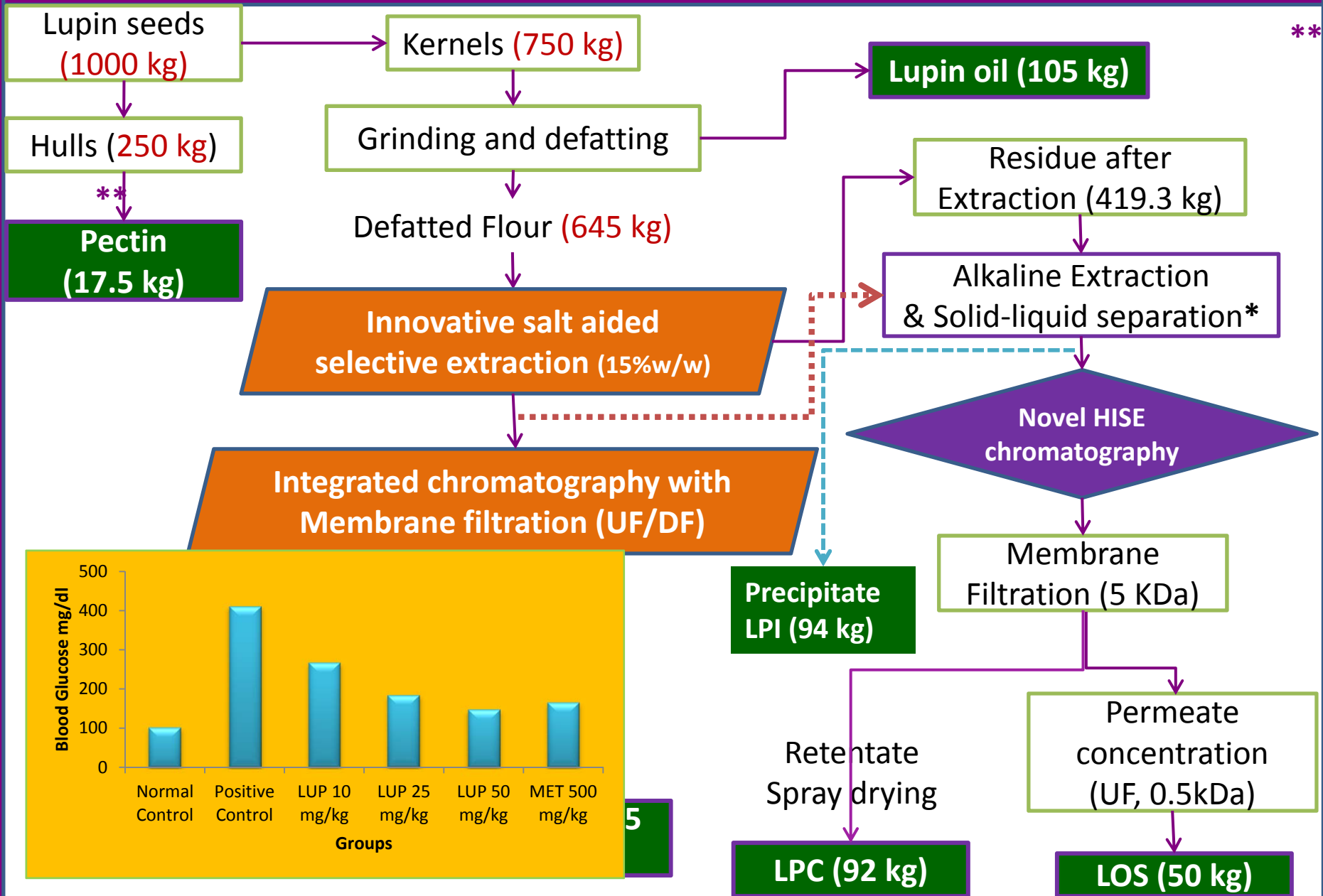
- **Ion exchange Chromatography**
- **Hydrophobic Interaction Chromatography**
- **Affinity Chromatography**

Physico Chemical Properties:

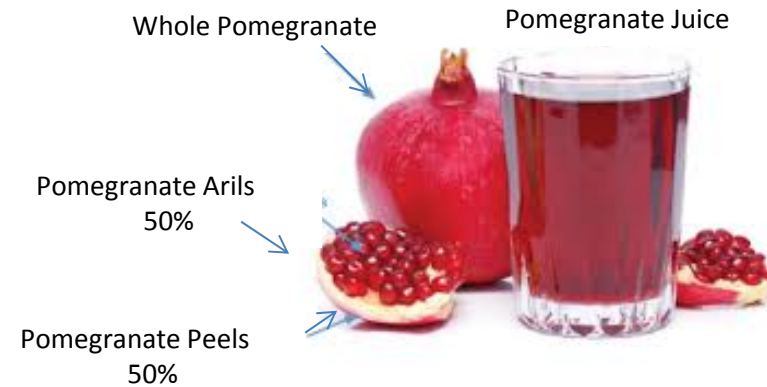
- ✓ **Solubility**
- ✓ **Binding Interactions**
- ✓ **Surface exposed hydrophobic residues**
- ✓ **Charged surface residues**
- ✓ **pI**
- ✓ **Size and Shape**



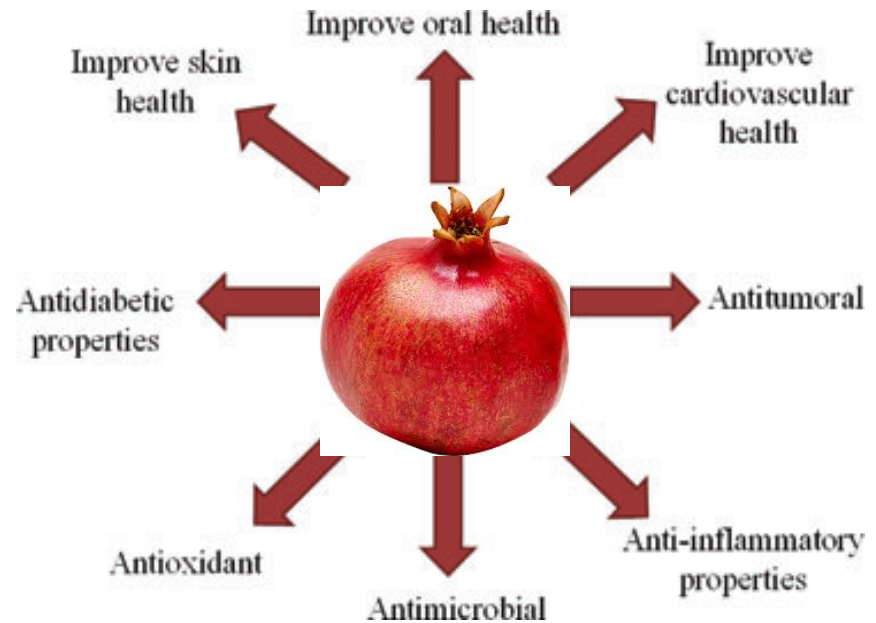
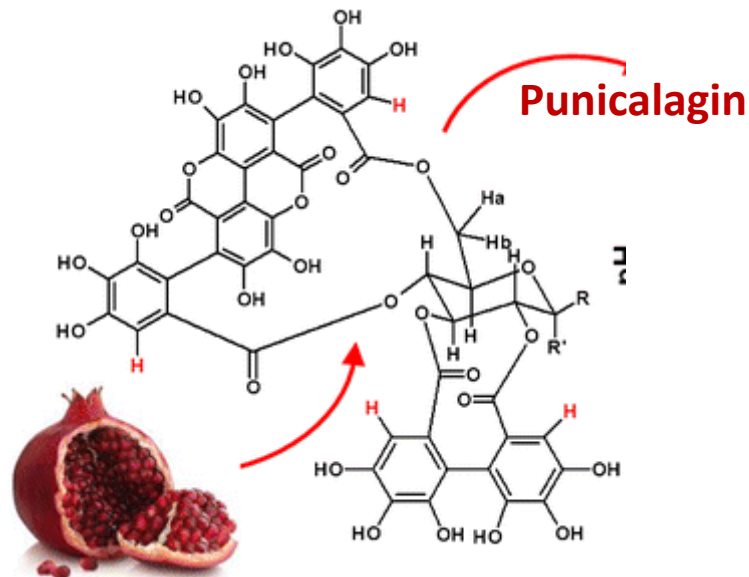
Overall Process with value addition



Punicalagin from pomogrenate

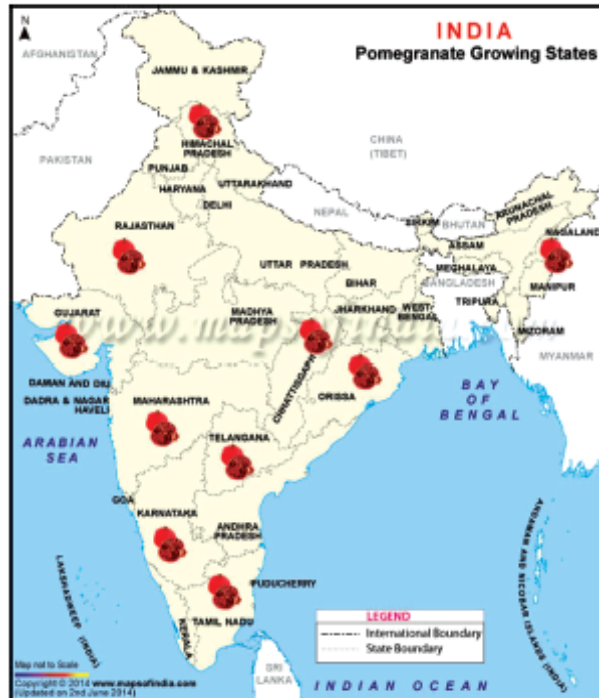


Composition of pomegranate peel powder

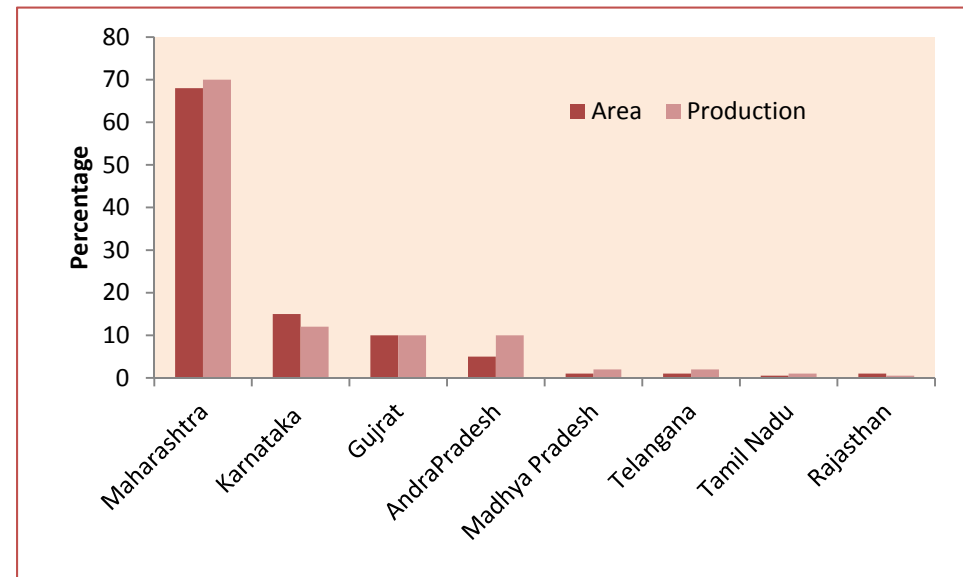


Pomogrenate production in India

- India ranks first in pomegranate production (Productivity= 7.4 tonnes/ hectare) in the world.



- Maharashtra, pomegranate basket of India alone shows productivity of 6.7 tonnes/ hectare



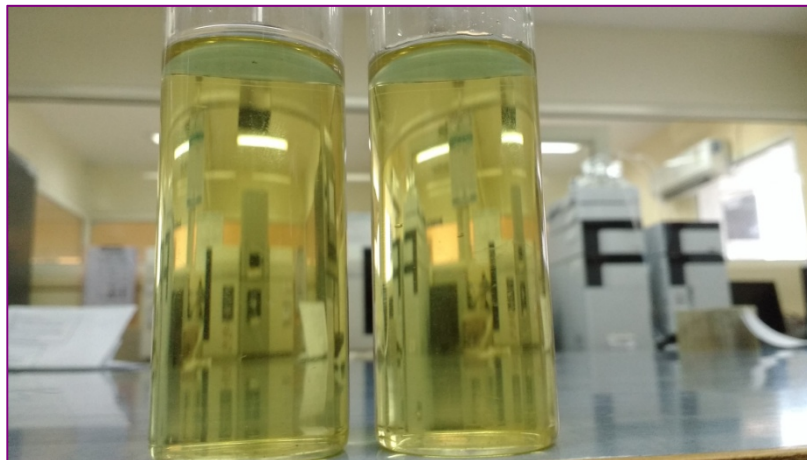
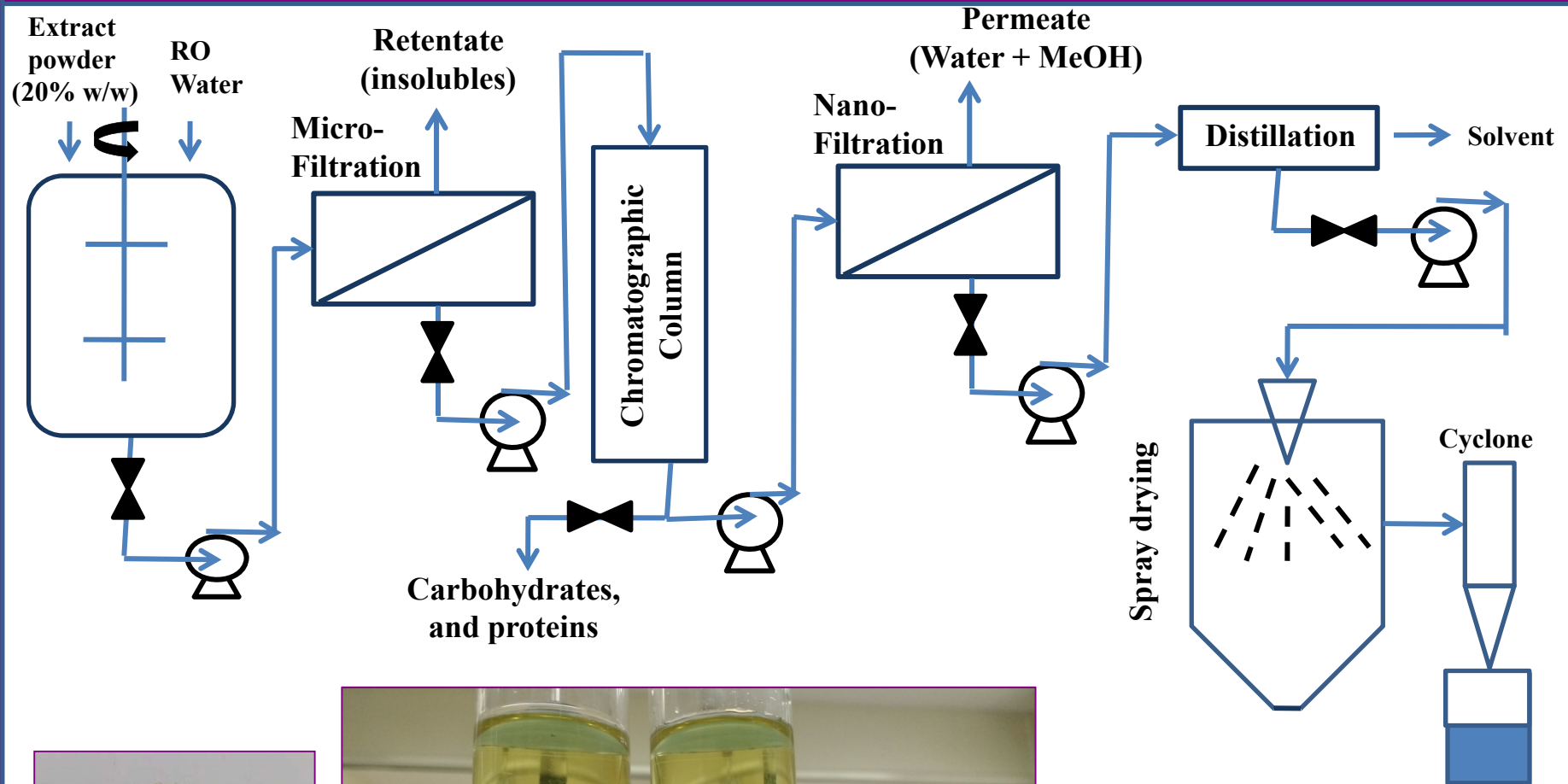
Value Addition:

- ✓ 50% peels from pomegranate juice processing Industry
- ✓ 20% post harvest losses- not suitable for export and domestic market

Objective:

To enrich punicalagin in pomegranate peel extract powder from 22% to >40% to meet commercial requirement

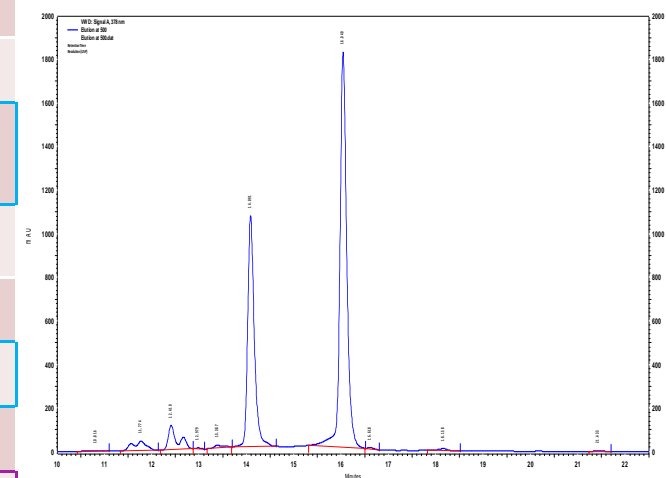
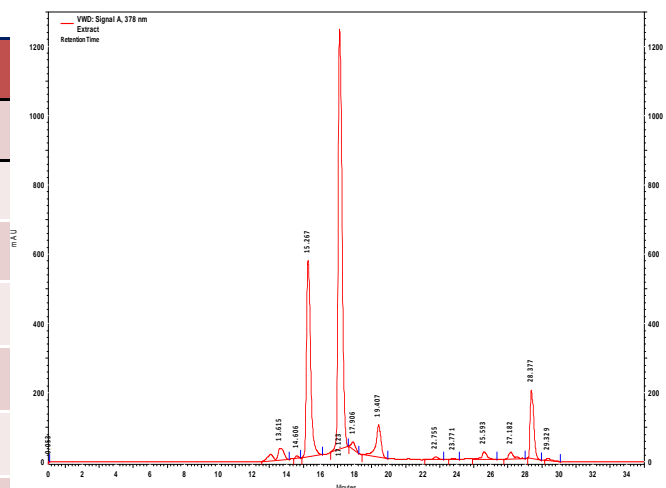
Process for Punicalagin



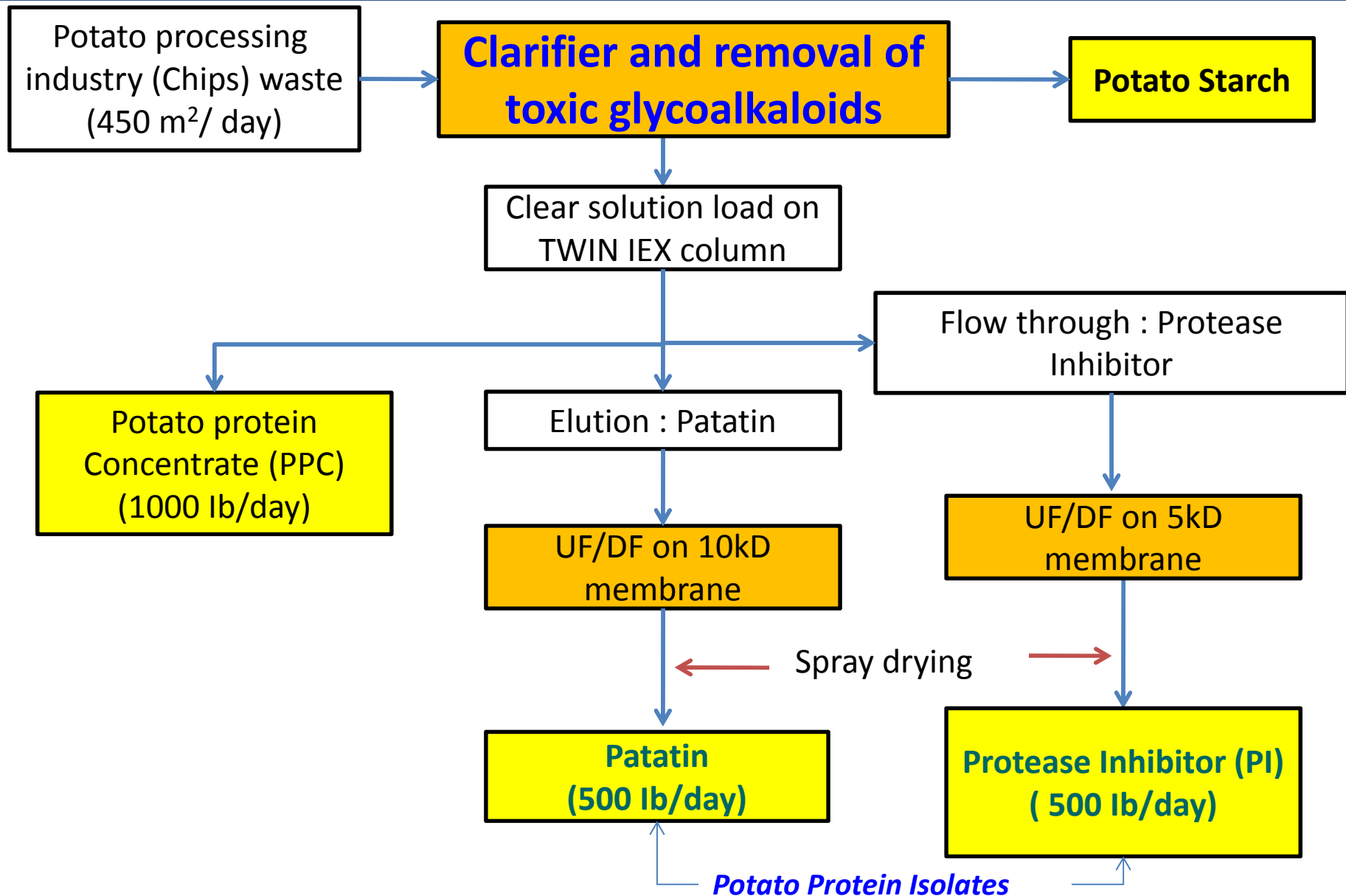
Punicalagin powder
Yield: 92%(w/w),
Assay Purity: ≥40%
(w/w)

Punicalagin Production - Scale Up

Conditions	Pilot Scale		
Loading Conditions			
Column Specifications	I.D.=20 cm , Ht.= 38.2 cm		
Bed Volume (lit.)	12		
Flow Rate (bv/hr)	0.8		
Assay Purity of Extract (%)	23.7		
Extract powder concentration	100 g/lit.		
Amount loaded (kg)	1 (237 g punicalagin)		
Volume loaded (lit.)	10		
Elution Conditions	% MeOH	BV	Amount (g)
Flow through (lit.)	-	10 lit.	0
Wash (water)	-	3	0
Elution	20	6	218
Regeneration	95	2	12
Results	Total Solid		Punicalagin
% Recovery	35		92
% Assay Purity	61		

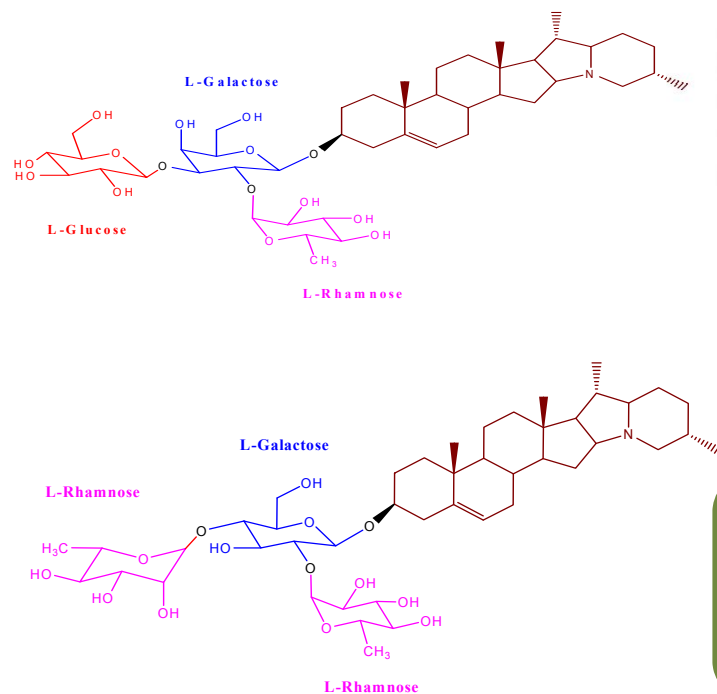


Natural Proteins – Potato proteins



Potato Proteins: Potential New Generation Nutraceutical

- Waste of potato processing industries: > 2.5 million m³
- Potato tuber juice (PTJ) contains ~0.4kg proteins per m³, consisting 40-45% patatin and 50-55% protease inhibitor proteins rich in lysine, having emulsifying properties, non-allergic
- Nutritional value greater than casein and equivalent to whole egg proteins
- Contains toxic glycoalkaloids i.e. α -Solanine and α -Chaconine >1000 ppm (on protein basis)



Potato proteins



Protease inhibitor (PI):
basic proteins, pI: 5.1-9.0,
2-22 kDa and 25-35 wt.%
of the potato proteins

High Mol. Wt.
(10-20%)

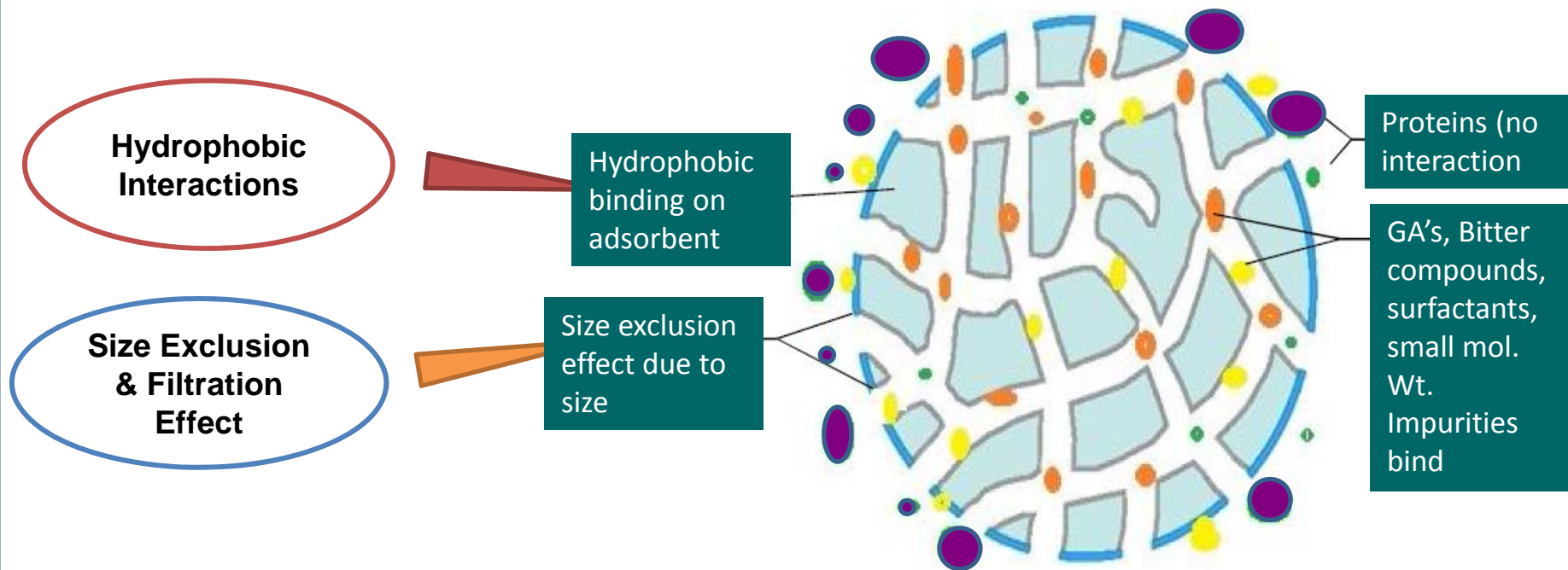
Patatin: Acidic glycoprotein, pI: 4-6
40-44 kDa and 40-50 wt.% of the potato
proteins, Lipolytic & acylase activity

- Acceptable limit of TGA is <100 ppm on protein basis

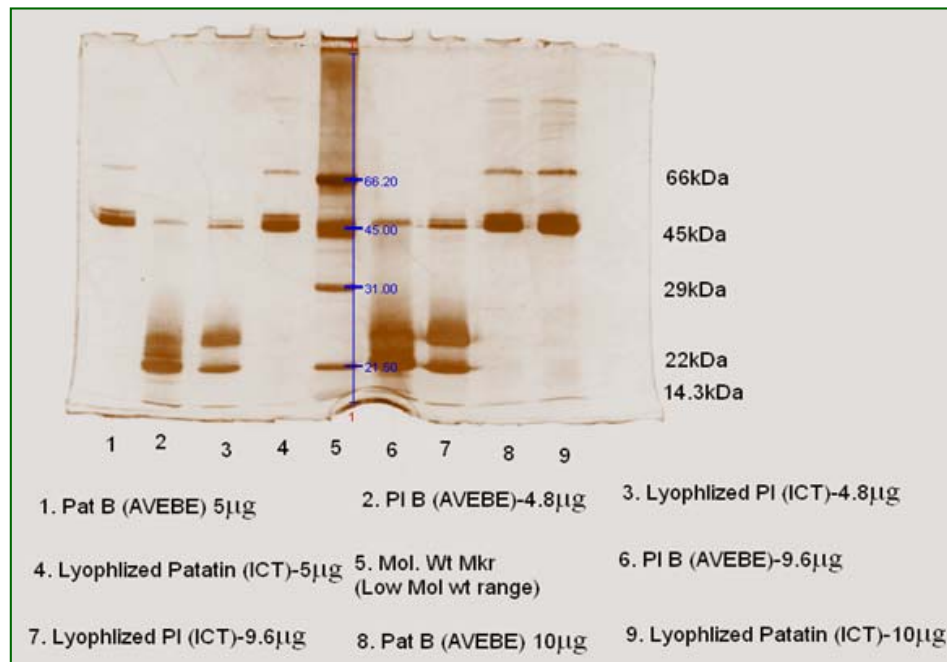
New Approach

HISEC: Hydrophobic interaction Size exclusion chromatography exploits *difference in hydrophobicity and size* of undesired small molecules and desired proteins for their filtration mechanism on the beads so as to have high capacity for unwanted small molecules and proteins are kept in flow through fractions.

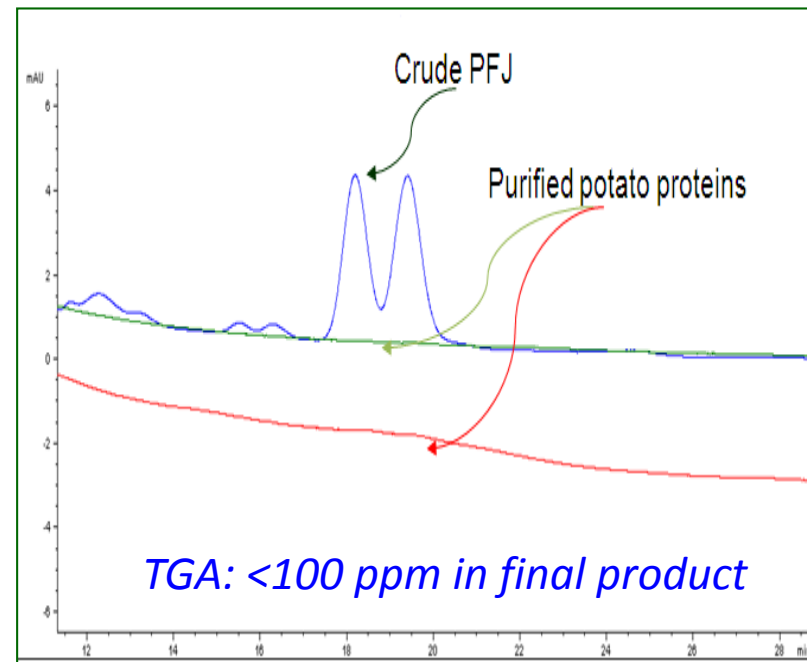
Here the adsorbent beads with narrow and small controlled pore size are used for achieving large loading of crude feedstocks.



Potato Proteins – Removal of toxic glycoalkaloids



NON-Reducing SDS-PAGE (12%) of patatin and protease inhibitor purified by DBT-ICT technology and potato proteins produced by AVEBE



No toxic glycoalkaloids were detected on RP-HPLC

*** HISE also applied to removal of isoflavones from soya proteins and soya milk**

Thank you ...



**Bioseparation technologies for
.....Health and Nutrition Mission**

**TAKE CARE OF
YOUR BODY.
IT'S THE ONLY
PLACE YOU HAVE
TO LIVE IN.**