HOHENHEIM UNIVERSITY
Institute of Food Science and Biotechnology
- Plant Foodstuff Technology
Stuttgart (Germany)

TALCA UNIVERSIDAD CHILE

Prof. Dr. habil. Dr. h.c. R. Carle

Elaboración de jugo de manzana y utilización comercial de sus subproductos

By-products of apple processing as a source of functional foods

Lecture at Talca University   March 27, 2012

Hohenheim University

Plant Foodstuff Technology (150d)

Prof. Dr. rer. nat. habil.
Dr. h.c. Reinhold Carle

New Processes for Improving Quality

- Development of innovative processes for the production of spices
- Recovery of anthocyanins and polyacetylenes from carrots and debittering of processed carrot products
- Recovery of protein from sunflower meal
- Pulsed electric fields in apple juice production
- Tropical fruit processing

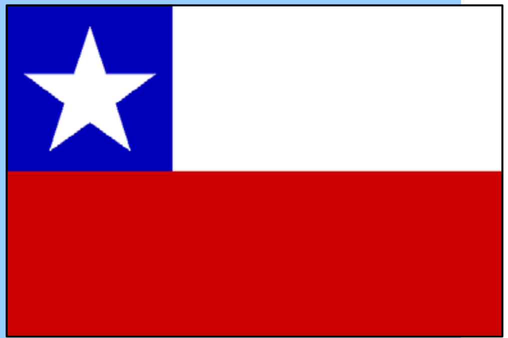
Development of Functional Food

- Recovery of techno- and biofunctional compounds from by-products of food processing, e.g. apple and grape pomace, mango peels
- Valorisation of organic wastes from technological processing in diets for livestock and humans
- Natural food colorants from fruits and vegetables (betalains, anthocyanins)
- Micro-encapsulation and characterization of bioactive compounds

Development of Methods – Quality and Authenticity Control

- Determination of fruit content in fruit products
- Determination of carotenoids and polyphenols in food supplements and food

APPLE COUNTRIES

FAO Statistic Division Centro de Pomáceas		ZMP-Marktbilanz OBST 2007
		
1 350 000	Apple Production [t]	1 600 000
11	Rank in the world	9
647 411	◄ Export [t] / Import [t] ►	698 311
35	… to Europe [%]	
32 000	… to Germany [t]	
→ Fresh fruit consumption		→ Juice processing

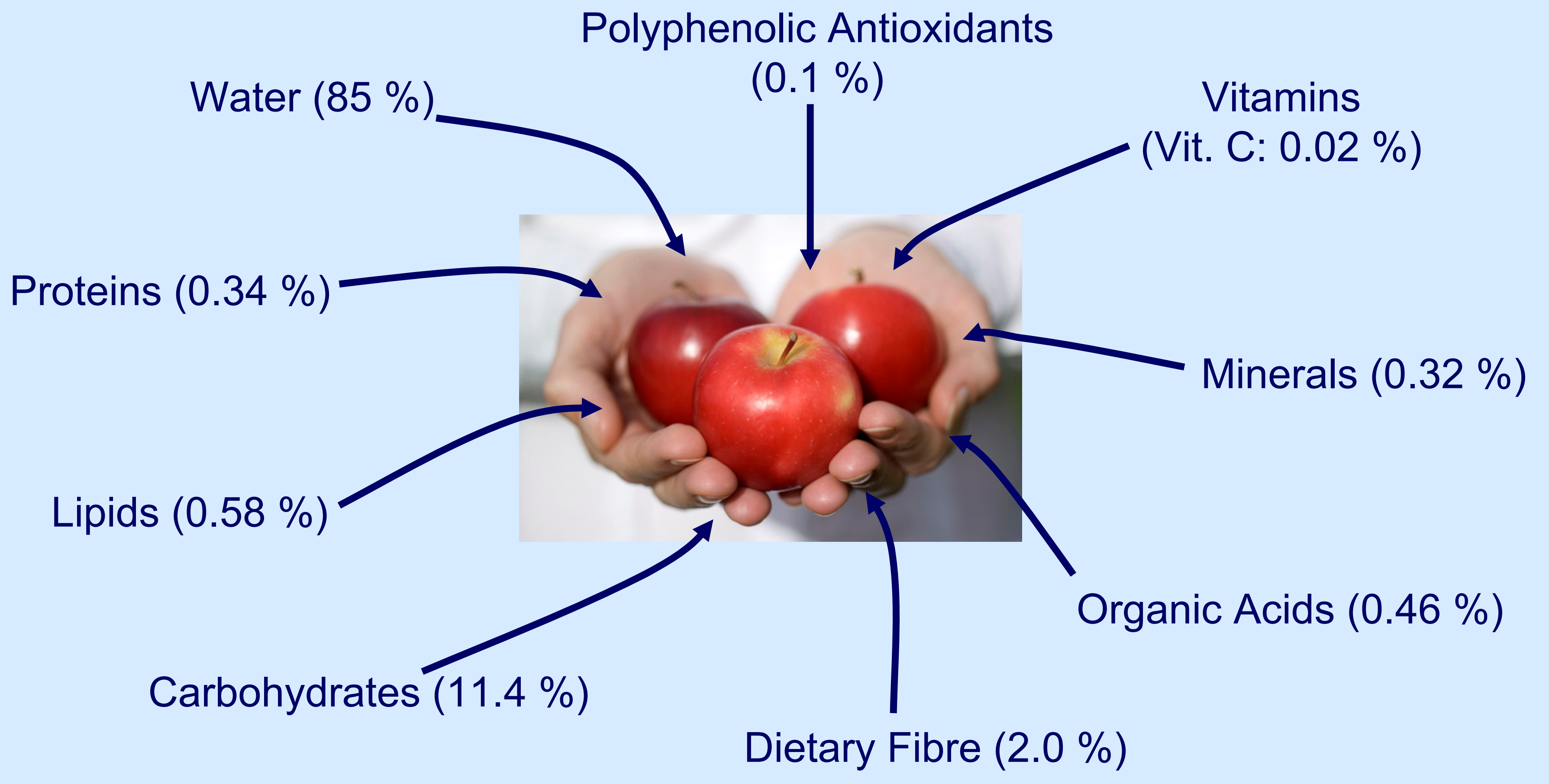
500 Mio. L/year
(670 000 t raw material)

Consumption:
40 L fruit juice/year,
12 L apple juice thereof

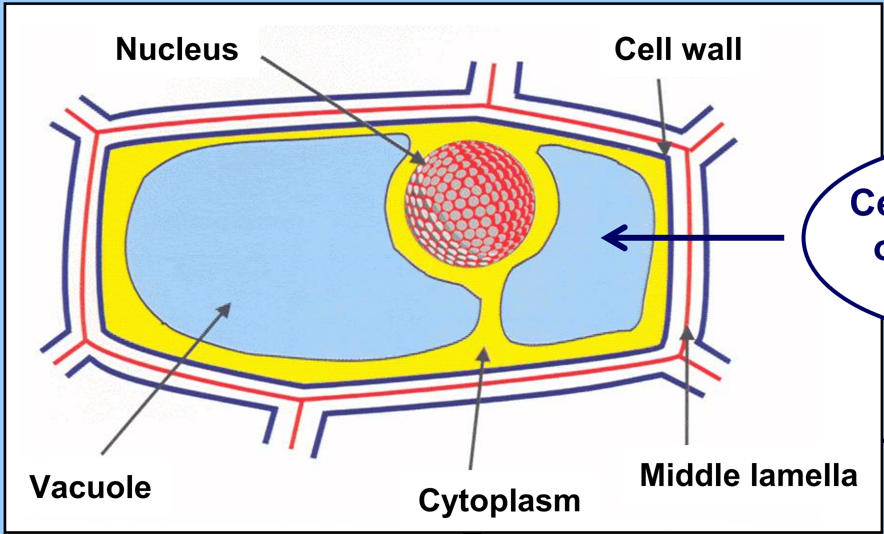
CIDER APPLE PRODUCTION

About 50 % of the raw material used in apple juice production are cider apples.

AVERAGE COMPOSITION OF APPLES



JUICE EXTRACTION: PRINCIPLES



Cell sap (sugars, organic acids, minerals)

Release of cell contents

Pressing

Mechanical damage of cell walls and membranes

by

- milling
- electroporation

↓

Pressing

Hot extraction

Heat denaturation of cell membranes

↓

Loss of semi-permeability

↓

Diffusion

Liquefaction

Enzymatic degradation of cell walls

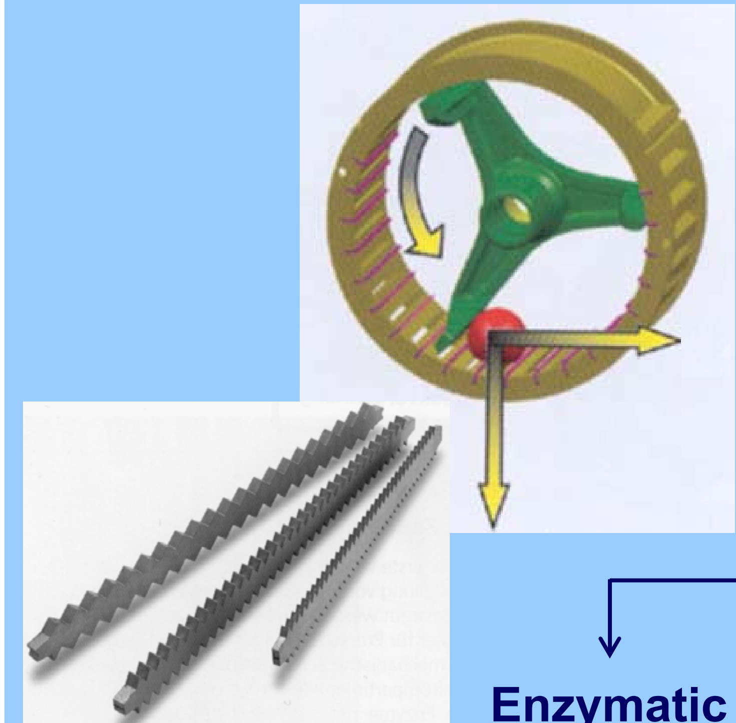
↓

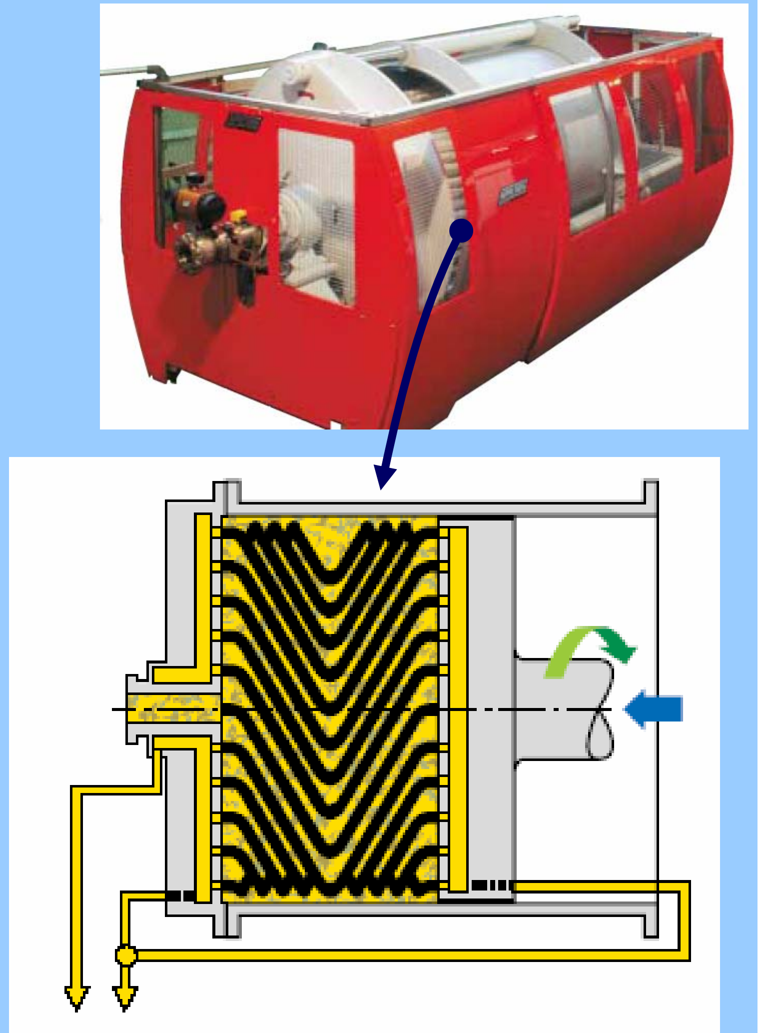
Sieving

(cores, seeds)

yield		
~ 75 - 80 %	~ 95 %	~ 100 %

APPLE JUICE PROCESSING





APPLES

↓

Milling

↓

MASH

→ Enzymatic treatment → Pressing

↓

Pressing → POMACE

↓

CLARIFIED JUICE

→ SINGLE STRENGTH JUICE

→ JUICE CONCENTRATE

A P P L E   P O M A C E

Quantity

APPLES
raw
700.000 t

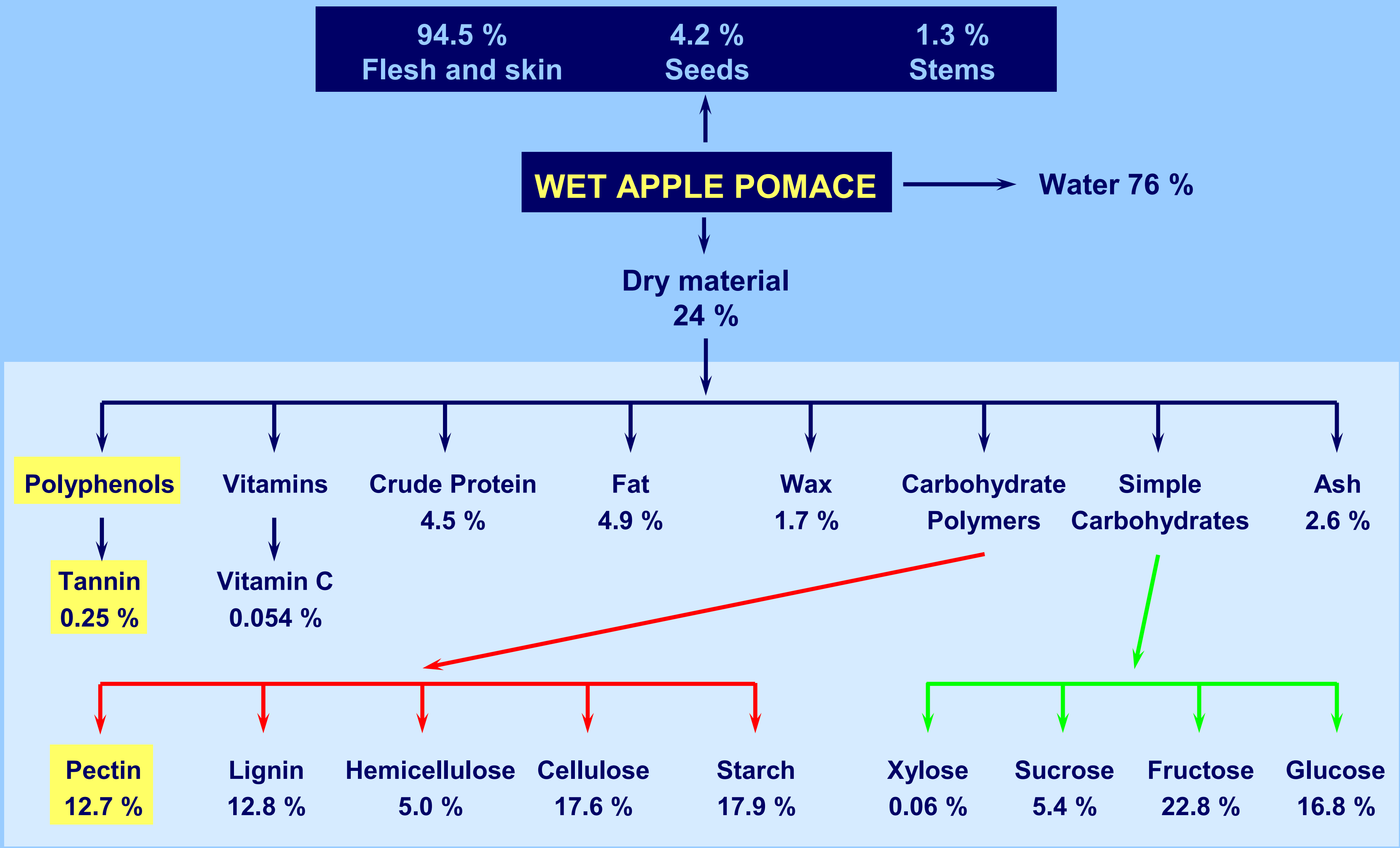
↓

Wet pomace
250.000 t
25 % DM

Waste disposal / utilization

- Composting
- Feeding of game animals
- Feeding of farm animals
- Waste combustion
- Landfilling
- Pectin recovery

AVERAGE COMPOSITION OF APPLE POMACE



The total comes to 125 %, because the compilation is based on many data varying over wide limits.

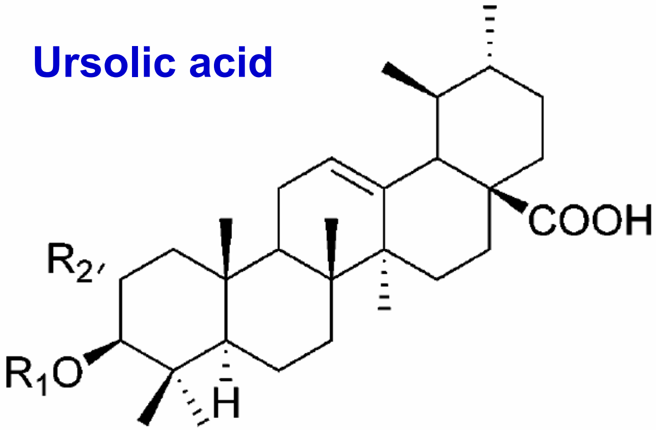
Kennedy, M. et al. In: Linskens, H.-F., Jackson, J.F. (eds.): Modern Methods of Plant Analysis. Vol. 20: Analysis of Plant Waste Materials. Springer (1999)

Apple peels

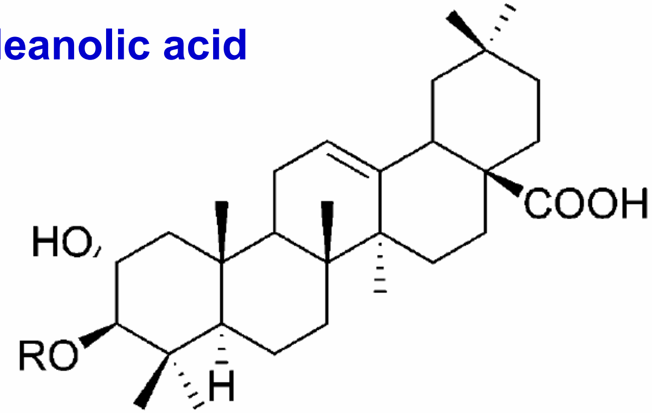
Epicuticular waxes:

Mixture containing hydrocarbons, alcohols, carbonyl compounds, fatty acids, …

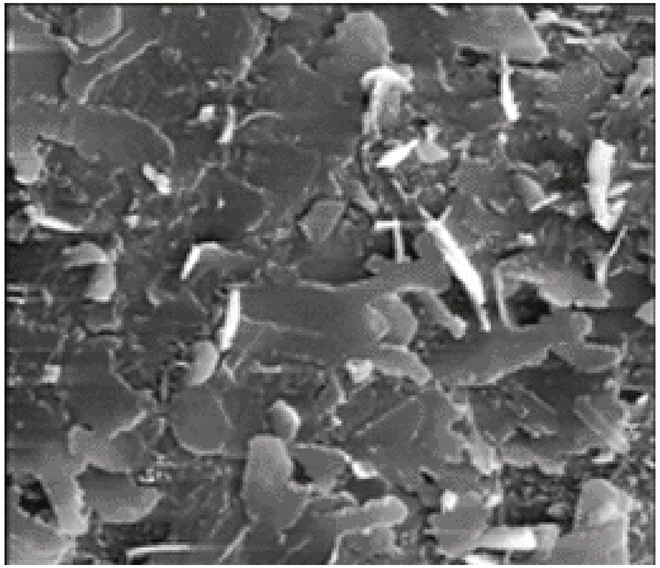
Ursolic acid



Oleanolic acid



Pentacyclic Triterpenoids



'Delicious' peel (x 2000)

Apple seeds

Proximate composition (%)

Component	Apple seed
Moisture	10.2±0.4
Crude fat	27.7±0.7
Crude protein	34.0±0.5
Carbohydrate	24.0±1.9
Ash	4.1±0.3

Polyphenol profile very restricted:

Phlorizin	75 %
Phloretin-2'-xyloglucoside	9 %
Quercetin-3-galactoside	6 %

Fatty acid composition (%)

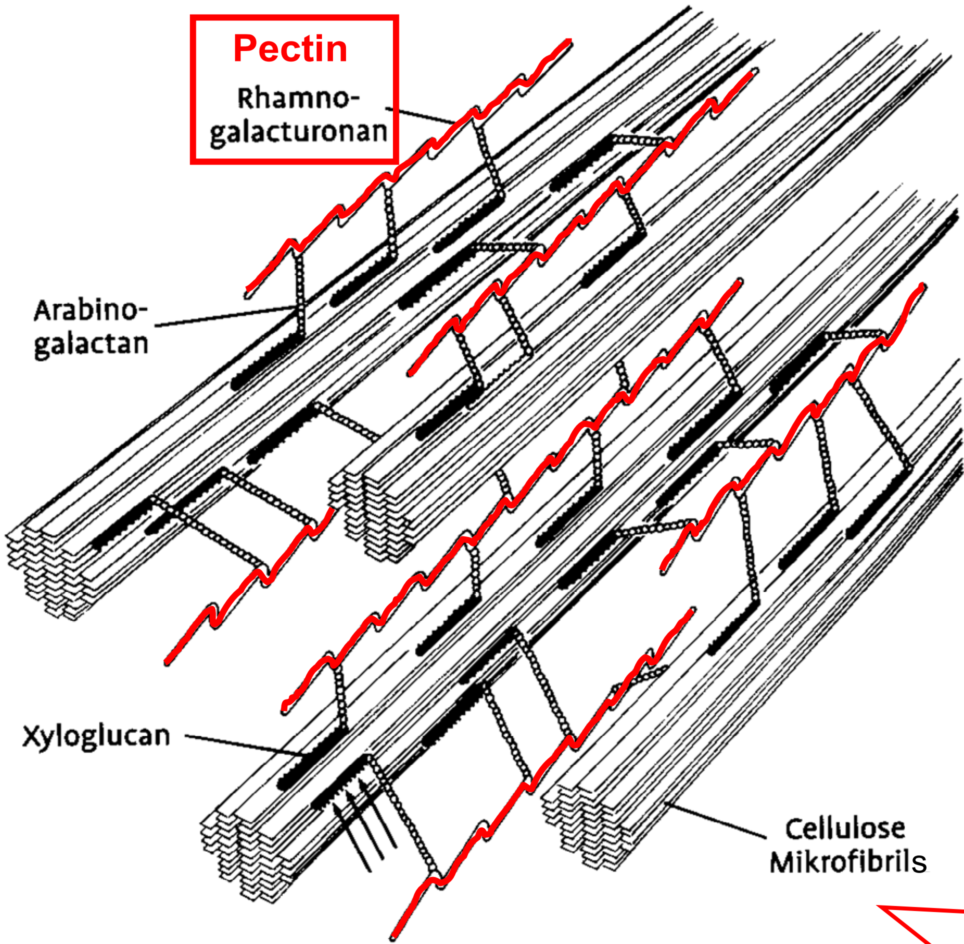
Fatty Acid	Apple Seed Oil
Palmitic	7.1±0.3
Stearic	2.4±0.2
Oleic	39.7±0.0
Linoleic	49.6±0.2
Linolenic	-
Saturated	9.5
Unsaturated	89.3

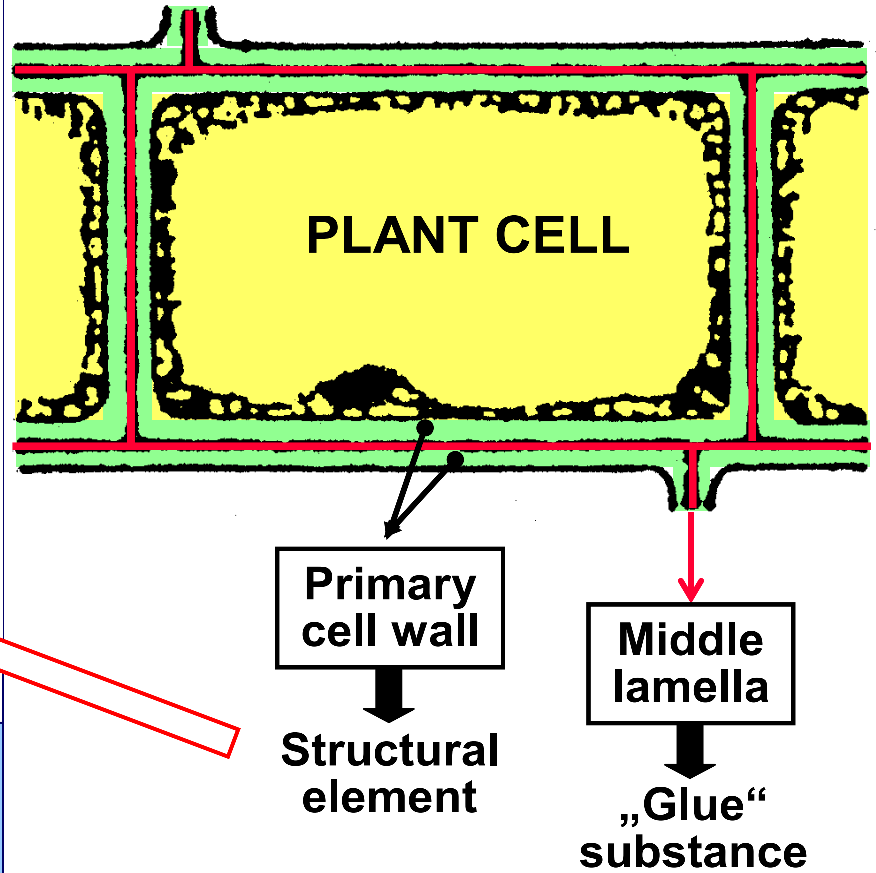
Application in cosmetics?
cf. almond or apricot seed oil

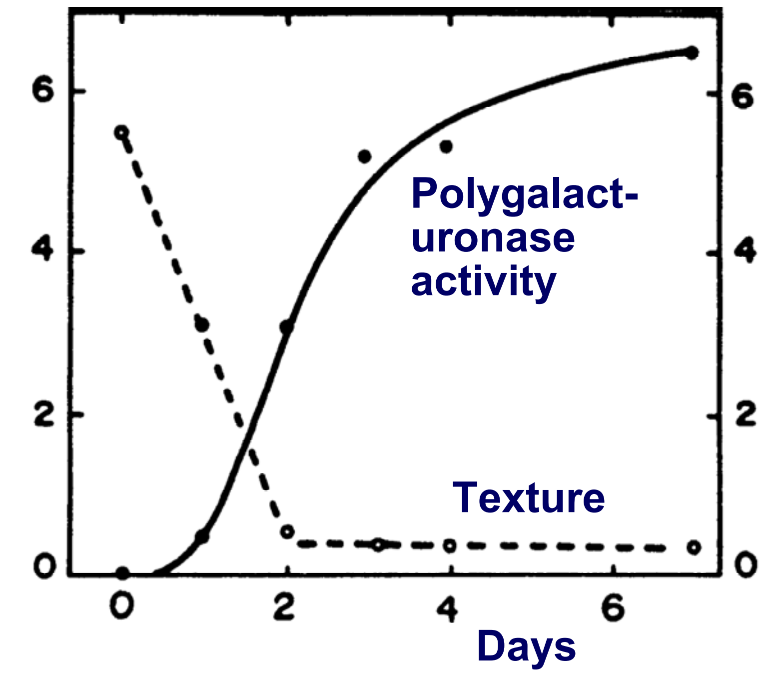
Li, Z., T. Yue, Int. J. Food Eng. 3(5), article 12 (2007)

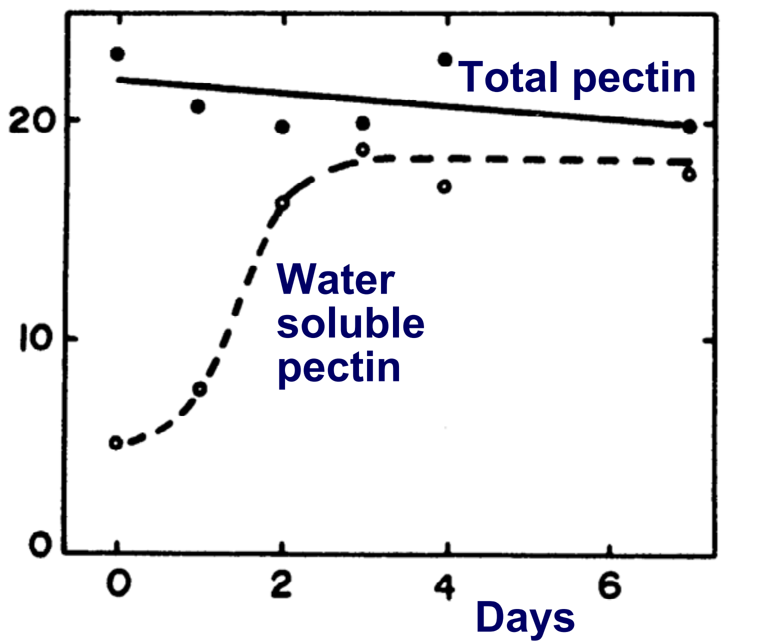
PECTIN

FUNCTION IN PLANT TISSUE









FRUIT RIPENING

Changes in

→ texture

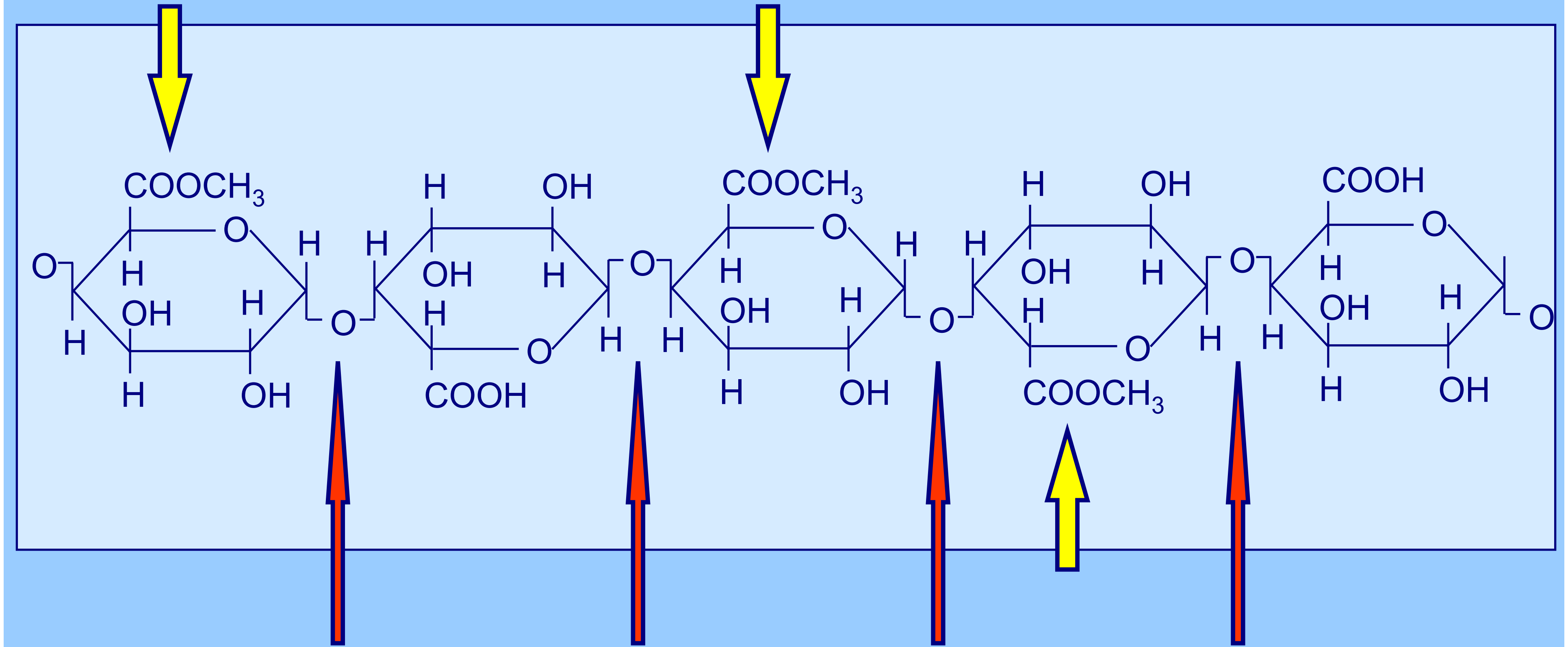
→ polygalacturonase activity

→ water soluble pectin

→ total pectin

PECTIN DEGRADATION

Synergism of pectic enzymes



1 **PECTIN ESTERASE** (Demethylation)

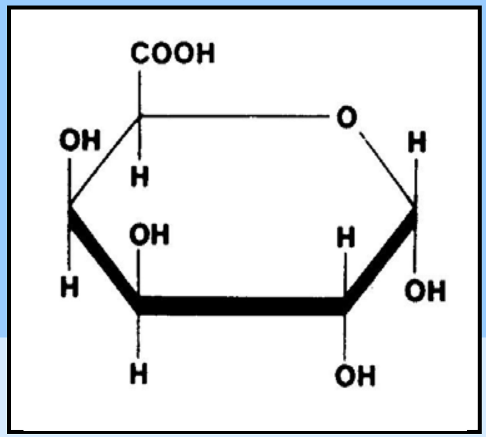
2 **POLYGALACTURONASE** (cleavage of chain)

Degree of Esterification (DE)

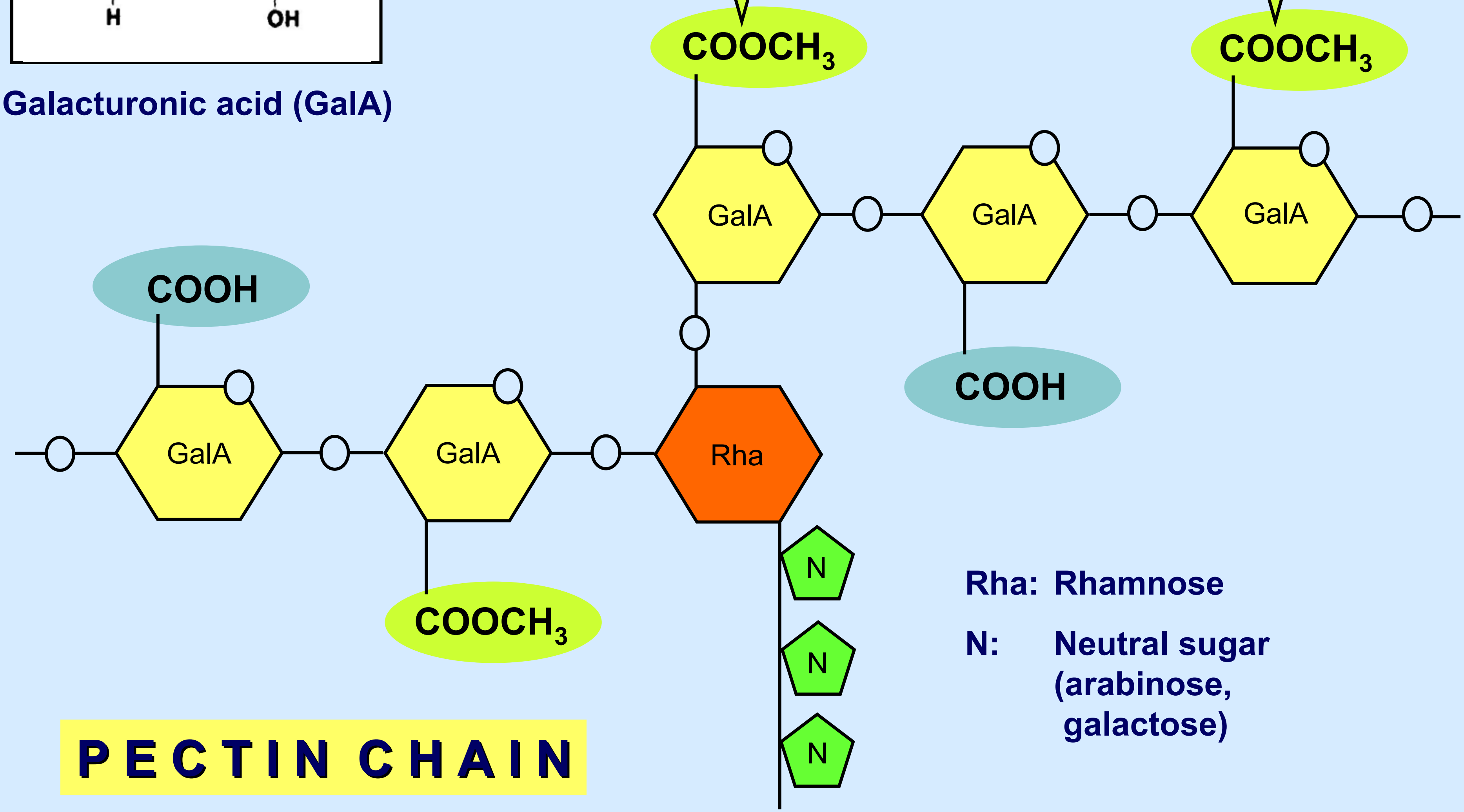
Ester linkage between acid (-COOH) and methanol (CH_3OH)

> 50 %: High esterified pectin

< 50 %: Low esterified pectin



Galacturonic acid (GalA)



Rha: Rhamnose

N: Neutral sugar (arabinose, galactose)

PECTIN CHAIN

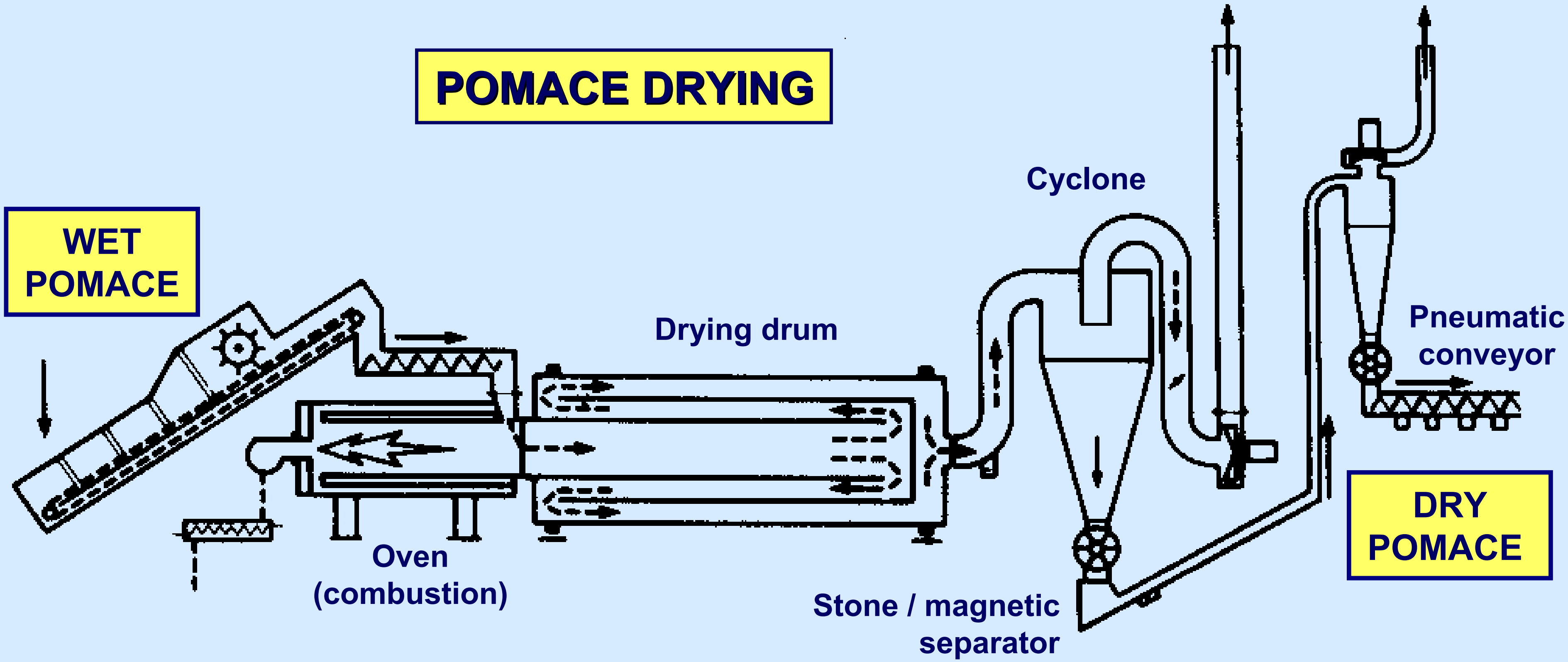
Pectin contents in plant foodstuffs

	Pectin (% fresh weight)
Apple	0.5 - 16
Currant	0.9 - 1.5
Potato	0.4 - 0.7
Carrot	0.8 - 2.2

Raw material for pectin extraction

	Pectin (% dry matter)
Apple pomace	10 - 15
Sugar beet	10 - 20
Sunflower heads	15 - 25
Citrus peels	20 - 35

POMACE DRYING

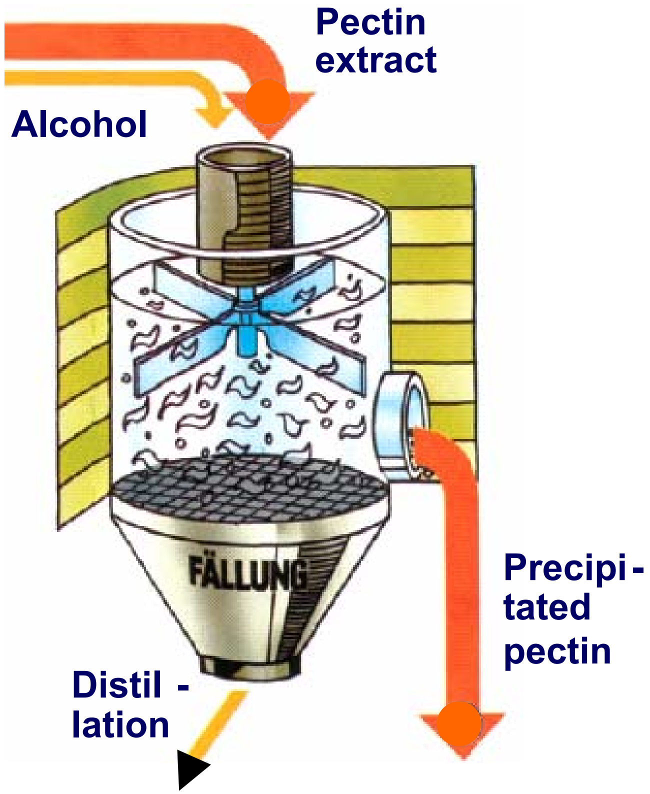


Endogenous pectinases

Oxidation (enzymatic browning)

Microorganisms (pectinases)

PECTIN EXTRACTION



Plant material

↓

Extraction

↓

Pectin extract ← Water, acid, heat

↓

Purification, concentration

↓

Precipitation

↓

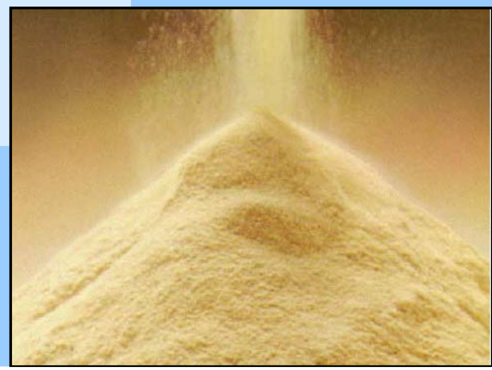
Pectin ← Alcohol

↓

Demethylation (opt.) ← Acid, alkali

↓

Drying, grinding, standardisation



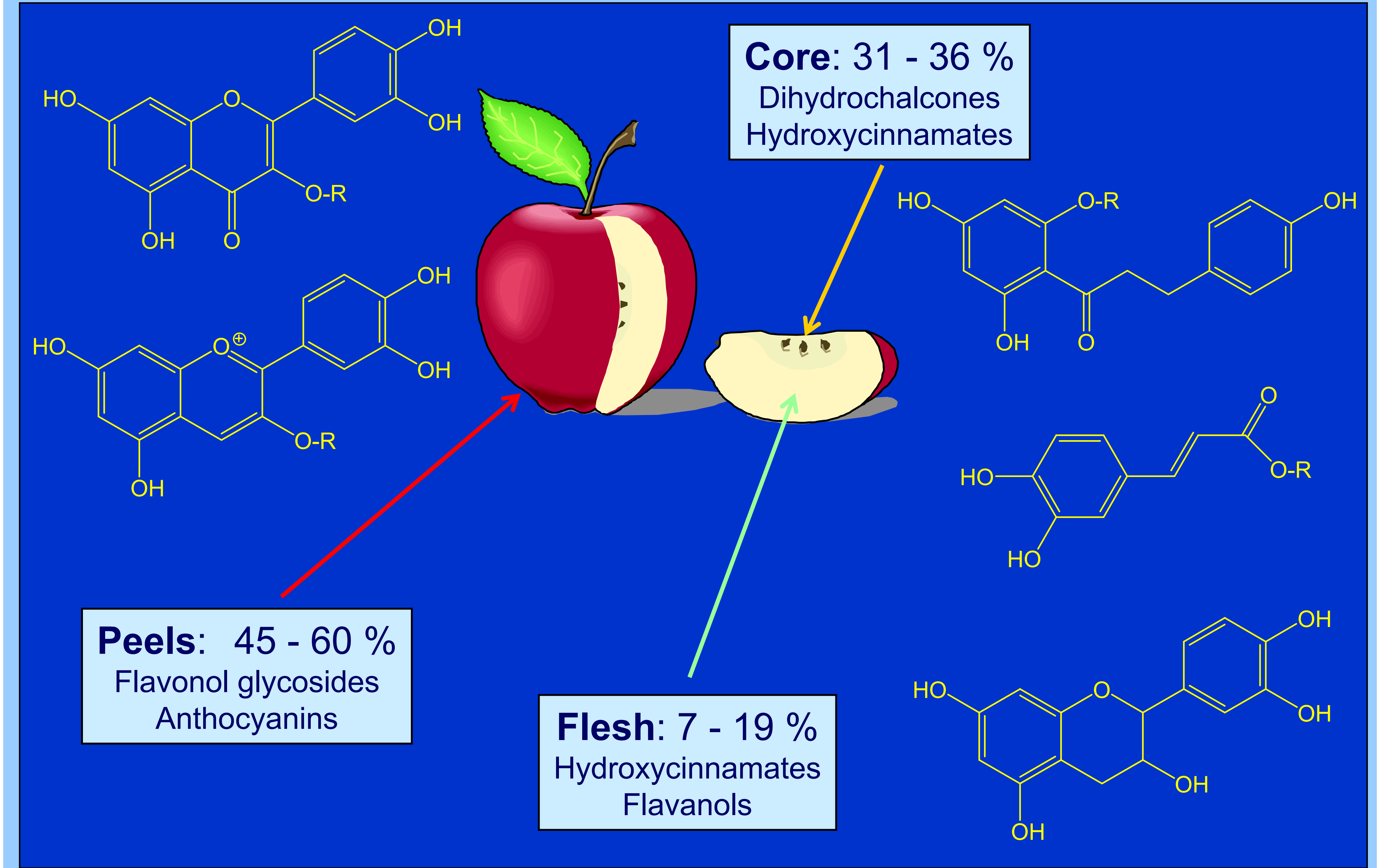
Different raw materials

↓

Colour of the extracted pectin

Apple pomace

Citrus peels



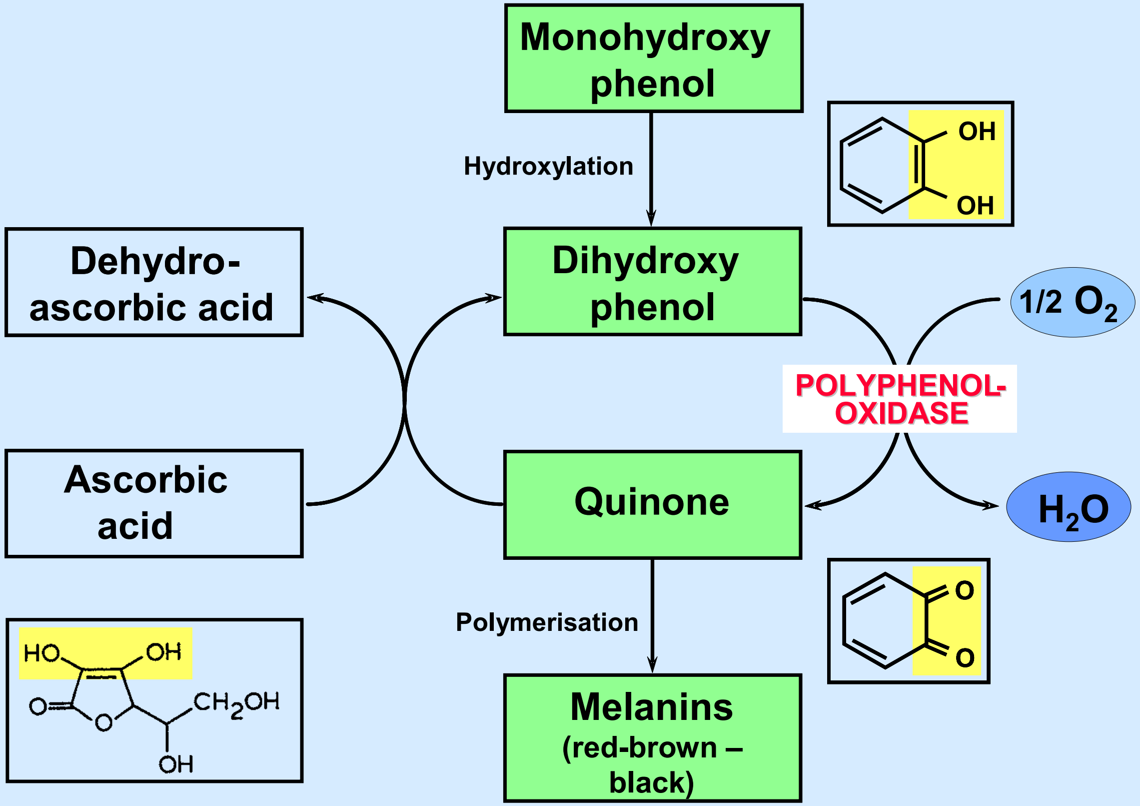
DISTRIBUTION OF PHENOLIC COMPOUNDS IN APPLE FRUITS

ENZYMATIC BROWNING

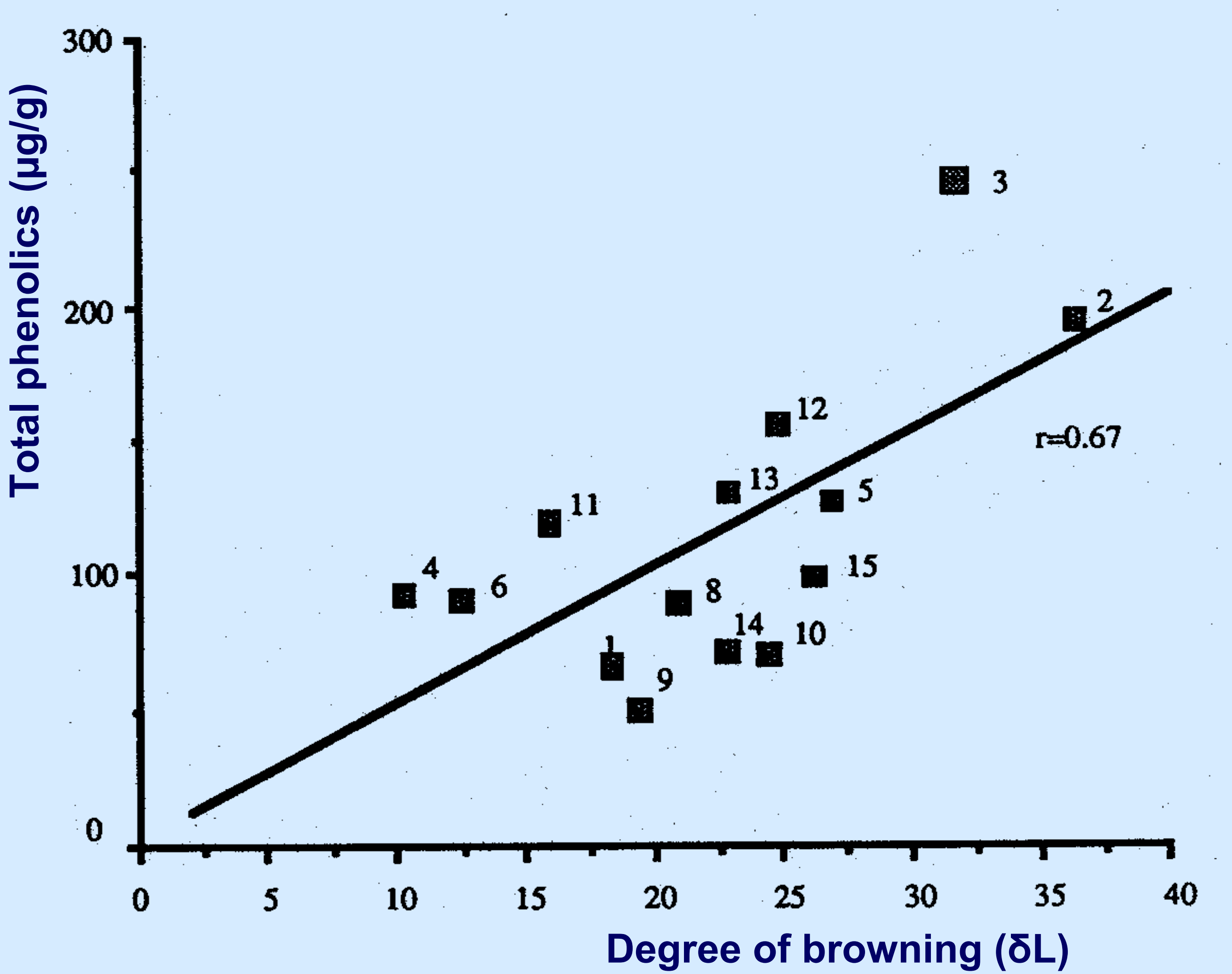
Brown discolouration following wounding (decompartmentalisation) of plant tissues

Prerequisites:

- Polyphenolics
- Oxygen
- Enzyme (polyphenoloxidase)

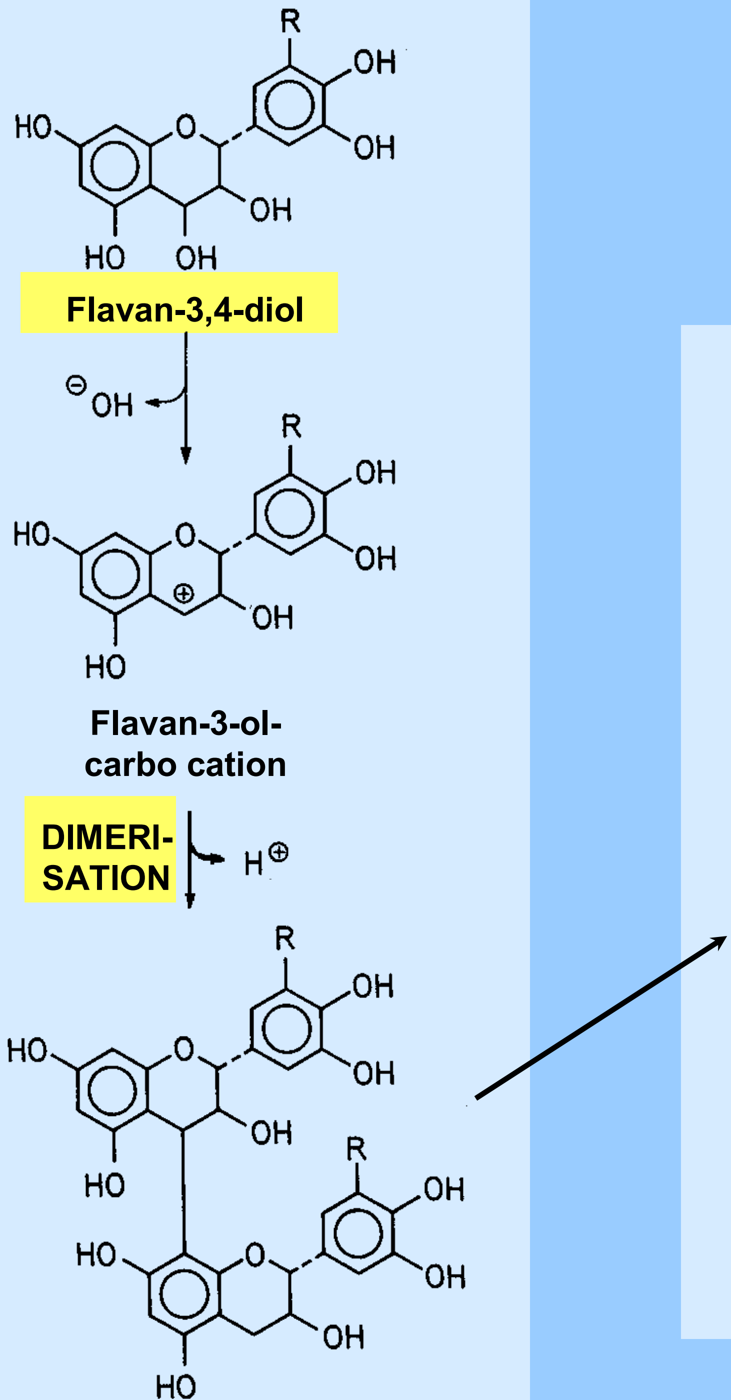


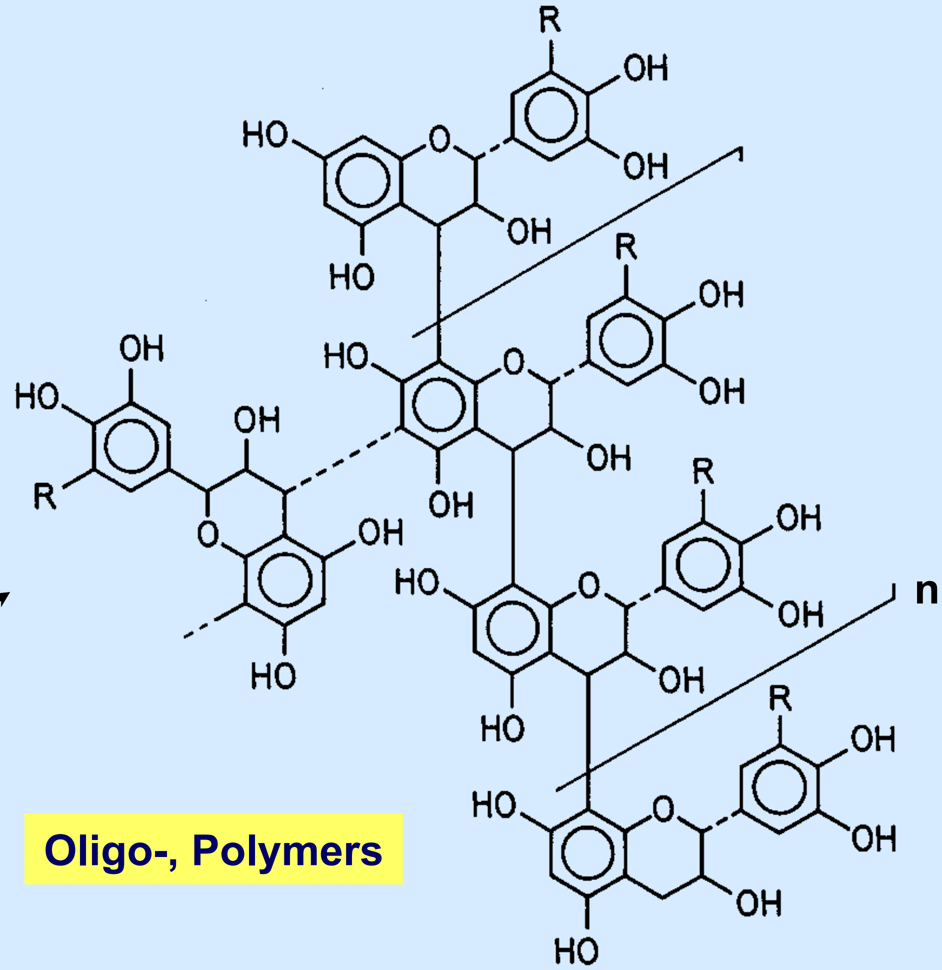
Relationship between browning and total polyphenols



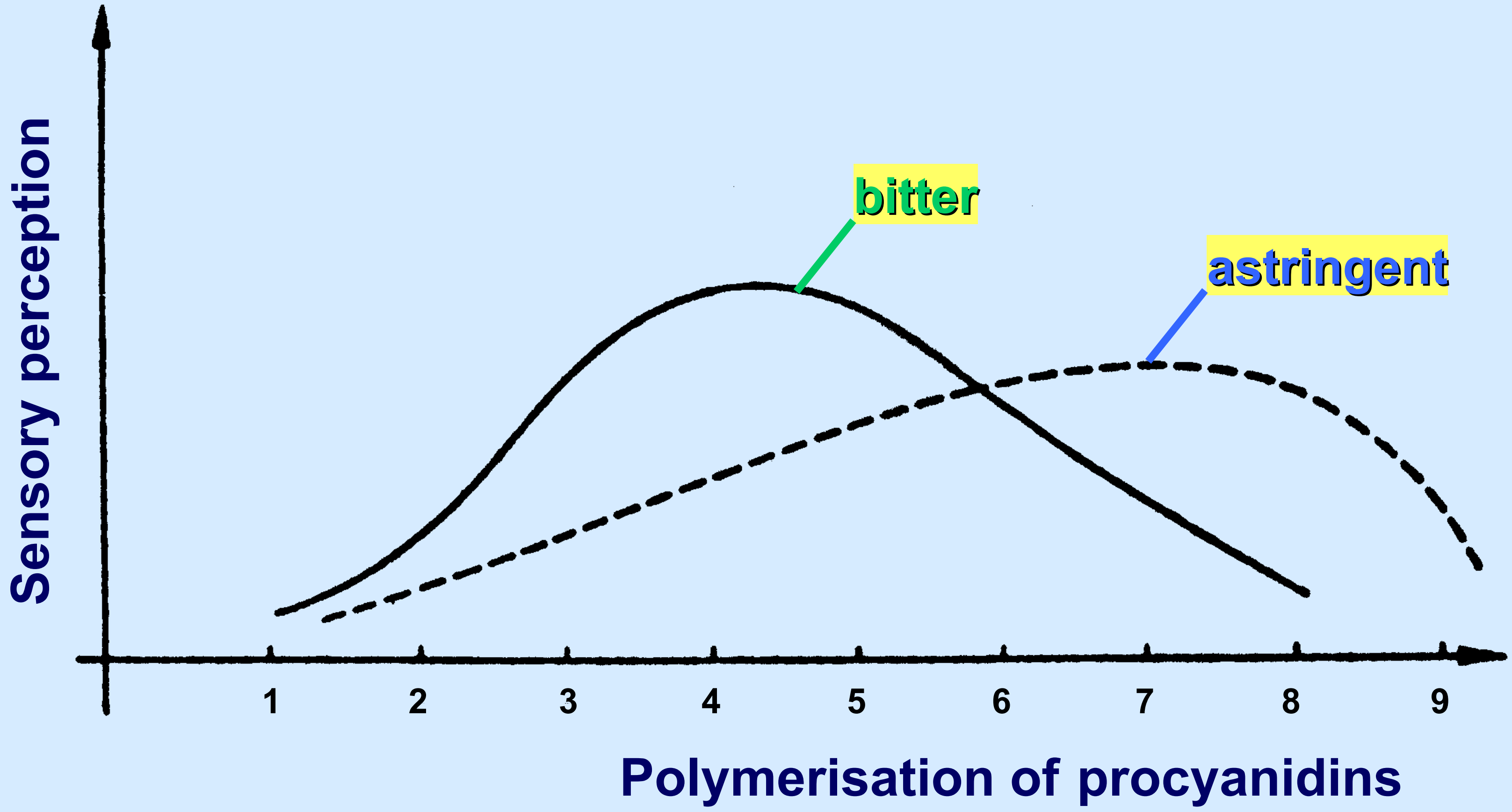
Lee, C.Y., in: Ho, C.-T.: Phenolic compounds ... (1992)

Formation of condensed or non-hydrolysable tannins





Correlation between sensory perception and polymerisation of procyanidins



Contents of phenolic compounds isolated from commercial apple pomace and apple juice samples

	Pomace (mg/kg dry matter)	Juice (mg/l)
Procyanidin B1	trace	2,3
Catechin	14	3,5
p-Coumaroyl glucose[1]	10	1,7
Chlorogenic acid	450	132
p-Coumaroyl quinic acid[1]	34	22,9
Procyanidin B2	47	9,1
Caffeic acid	8	5,1
Epicatechin	77	12,1
p-Coumaric acid	3	0,6
Quercetin 3-galactoside	360	1,5
Quercetin 3-glucoside	130	1,0
Quercetin 3-xyloside	160	0,9
Quercetin 3-rhamnoside	230	3,4
Phloretin 2'-xyloglucoside[2]		11,4
Phloridzin	910	23,8
Quercetin	67	1,2
Phloretin	trace	

[1-2] tentatively identified; calculated as [1] coumaric acid, [2] phloridzin

Processing

Apple juice

6 - 17 % antioxidant activity of fresh apples

Loss

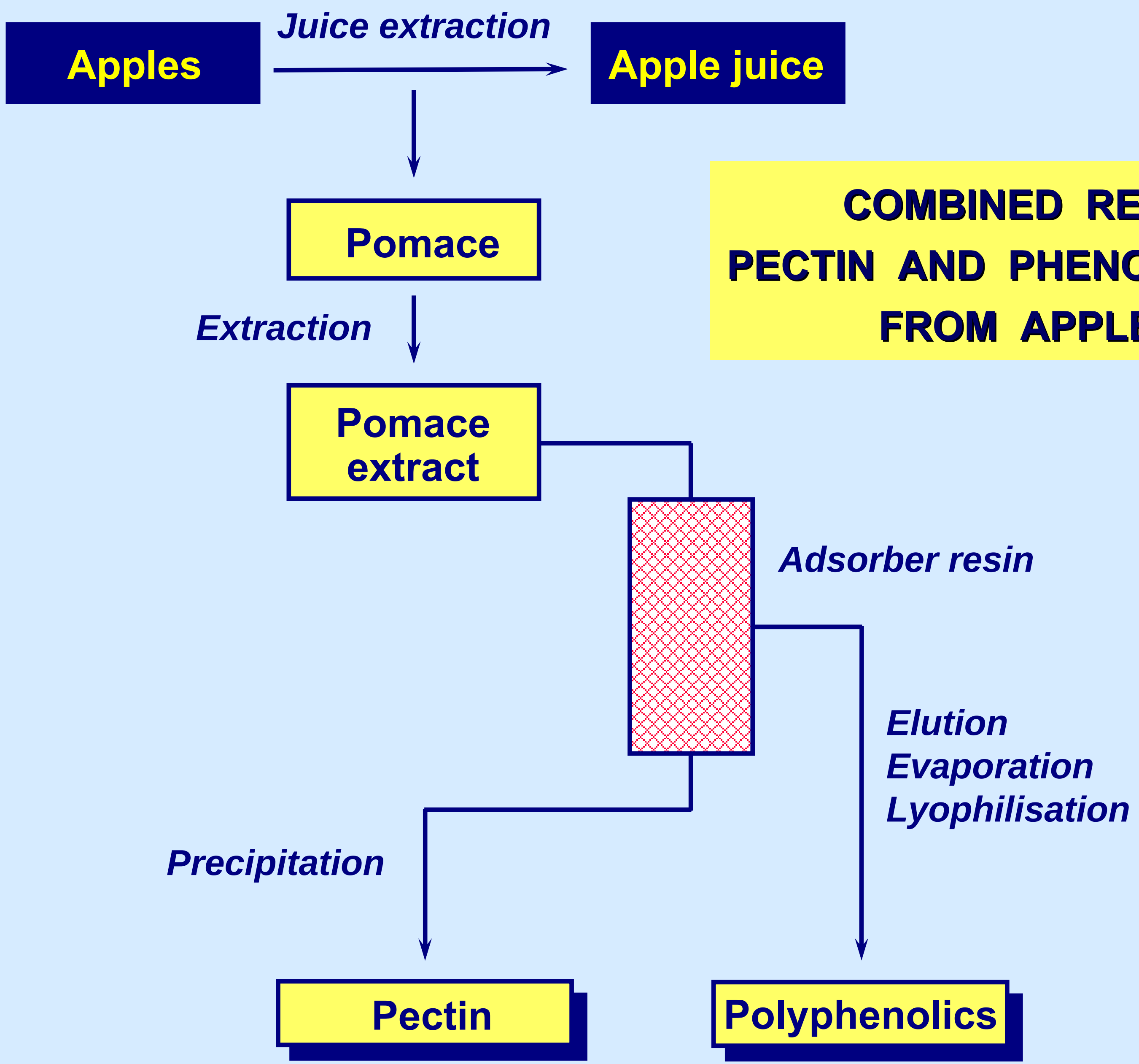
20 - 40 % of antioxidant activity of freshly pressed juice

Apple pomace

> 50 % of antioxidant activity

Kammerer, D.R., The Horticulture and Food Research Institute, New Zealand

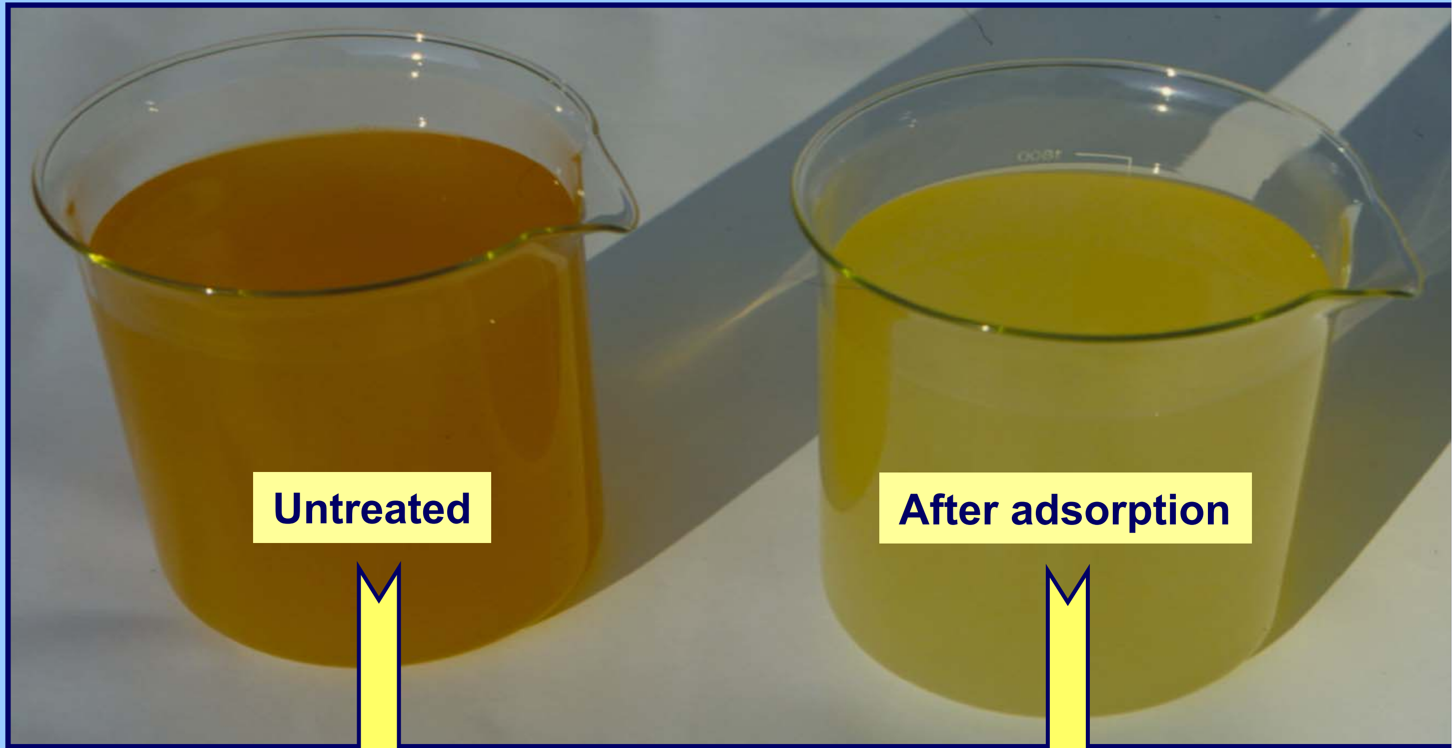
COMBINED RECOVERY OF PECTIN AND PHENOLIC COMPOUNDS FROM APPLE POMACE



Carle, R. et al.: DE 10018533 (31.10.2002)
Schieber, A. et al., Innov. Food Sci. Emerg. Techn. 4, 99-107 (2003)

IMPROVEMENT OF PECTIN QUALITY

Decolorisation

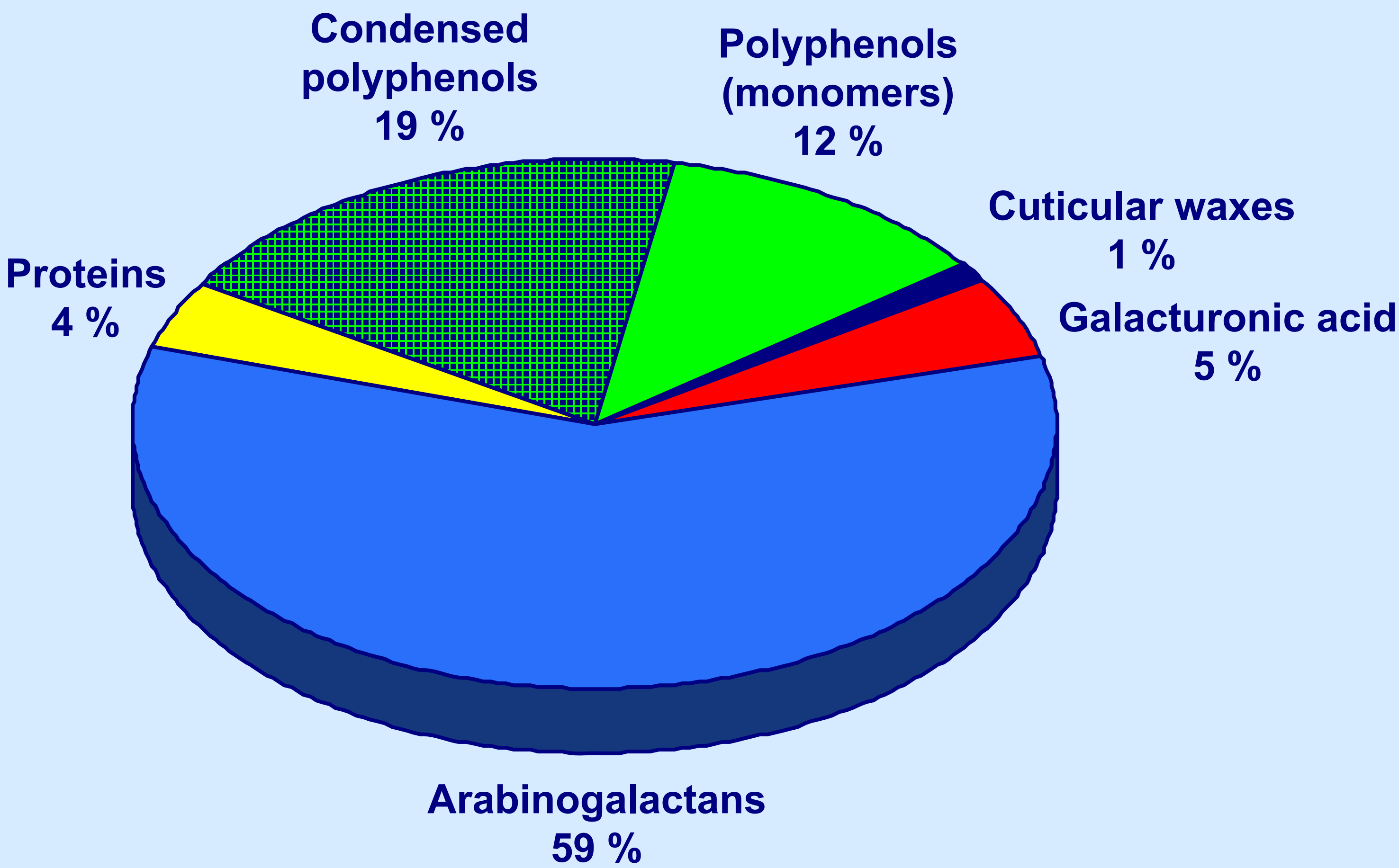


Gelling properties

		Untreated	After adsorption
• Galacturonic acid	[%]	50,9	54,4
• Breaking strength (HPE)	0,25 %	341	435
	0,36 %	535	756

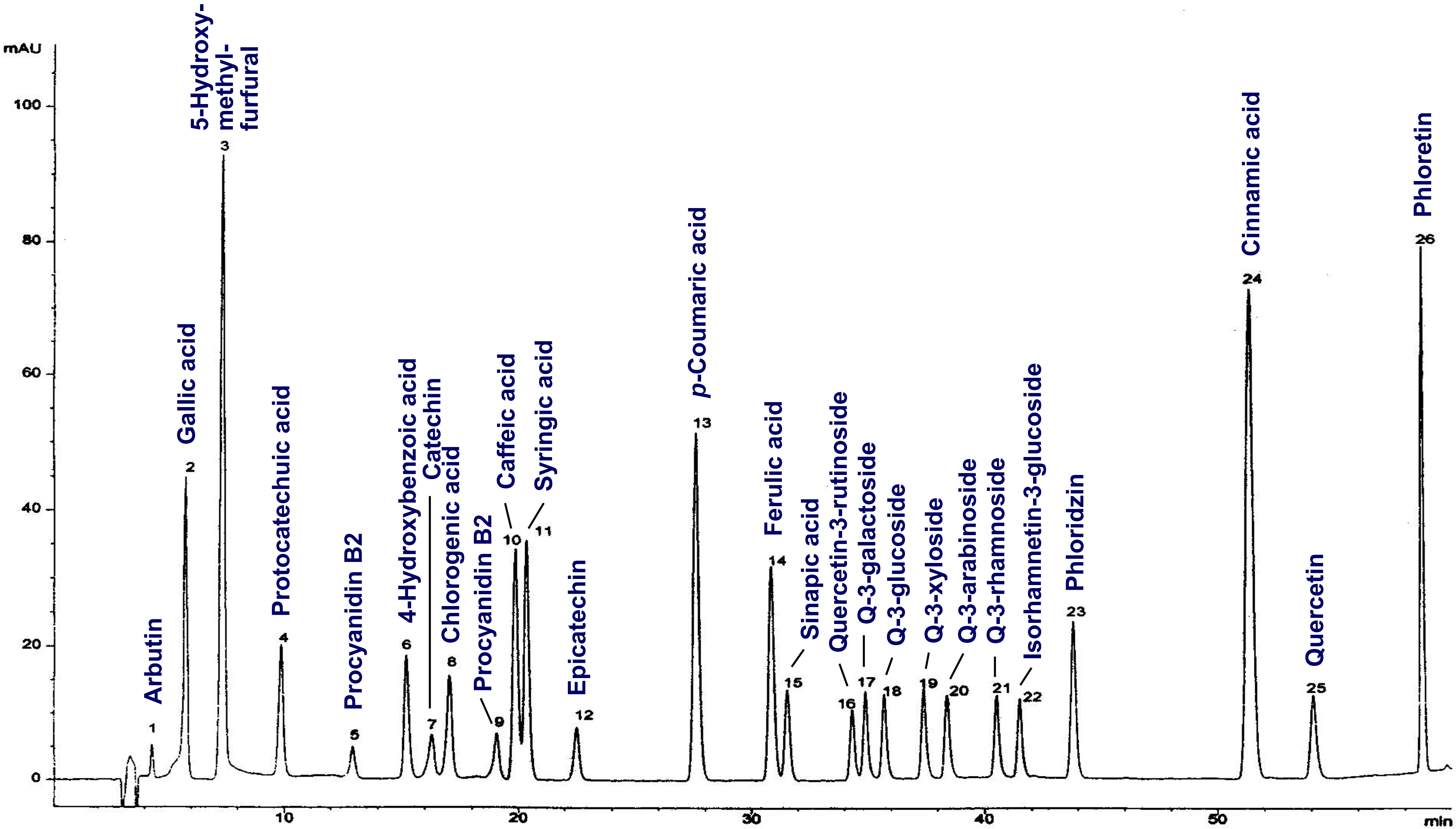
Schieber, A., Hilt, P., Streker, P., Endress, H.-U., Rentschler, C., Carle, R. (2003) Innovative Food Science and Emerging Technologies 4, 99-107

COMPOSITION OF AN APPLE POMACE EXTRACT



Phenolic compounds: Separation of a standard mixture (RP-HPLC, 280 nm)

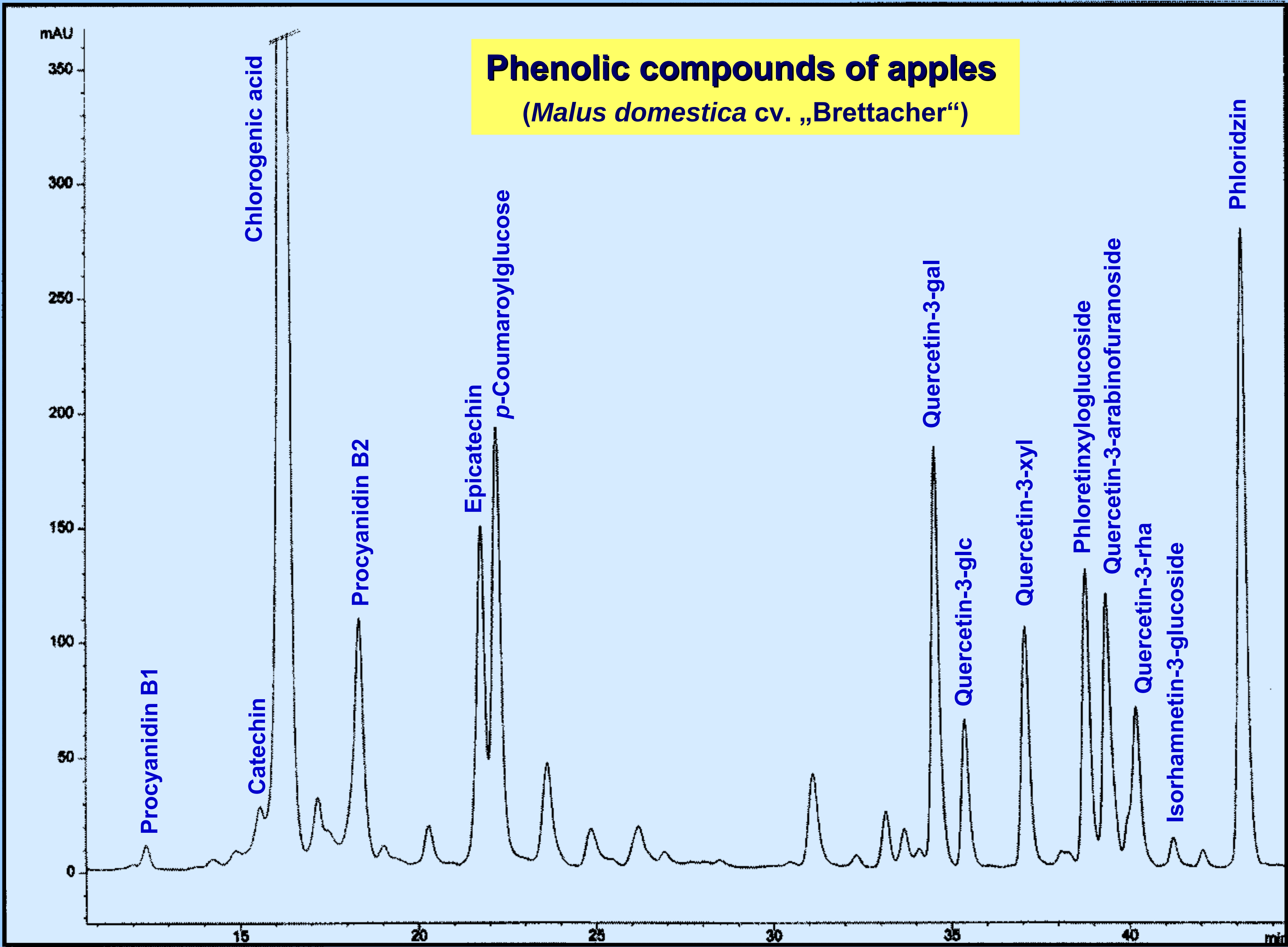
Column: Aqua 5 µm C18 (250 mm x 4,6 mm i. D.), Phenomenex



Schieber, A., Keller, P., Carle, R. (2001) Journal of Chromatography A 910, 265-273

Phenolic compounds of apples

(*Malus domestica* cv. „Brettacher“)



HPLC separation of the extract (280 nm)

Composition of the phenolic fraction obtained from an apple pomace extract

[mg/g lyophilisate]

Chlorogenic acid	14,3	Epicatechin	9,3
p-Coumaroylquinic acid	1,8	Procyanidin B2	9,3
p-Coumaric acid	0,5	Catechin	2,4
Ferulic acid	0,4		
Quercetin-3-galactoside	11,4	Phloridzin	40,4
Q-3-rhamnoside	4,7	Phloretin xyloglucoside	8,0
Q-3-glucoside	3,9		
Q-3-xyloside	1,8	Quercetin	6,5
Q-3-rutinoside	1,3	Phloretin	0,5
Q-3-arabinoside	1,1		

Schieber, A., Hilt, P., Streker, P., Endress, H.-U., Rentschler, C., Carle, R. (2003) Innovative Food Science and Emerging Technologies 4, 99-107

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Schieber, A., Hilt, P., Streker, P., Endress, H.-U., Rentschler, C., Carle, R. (2003) Innovative Food Science and Emerging Technologies 4, 99-107

Influence of drying on the content of phenolic components in apple pomace

[mg/kg dry matter]

	POMACE WET [1]	POMACE DRY [2]
Flavonols	633	673
Flavanols	406	318
Dihydrochalcones	867	861
Hydroxycinnamates	502	562
Total	**2 408**	**2 414**

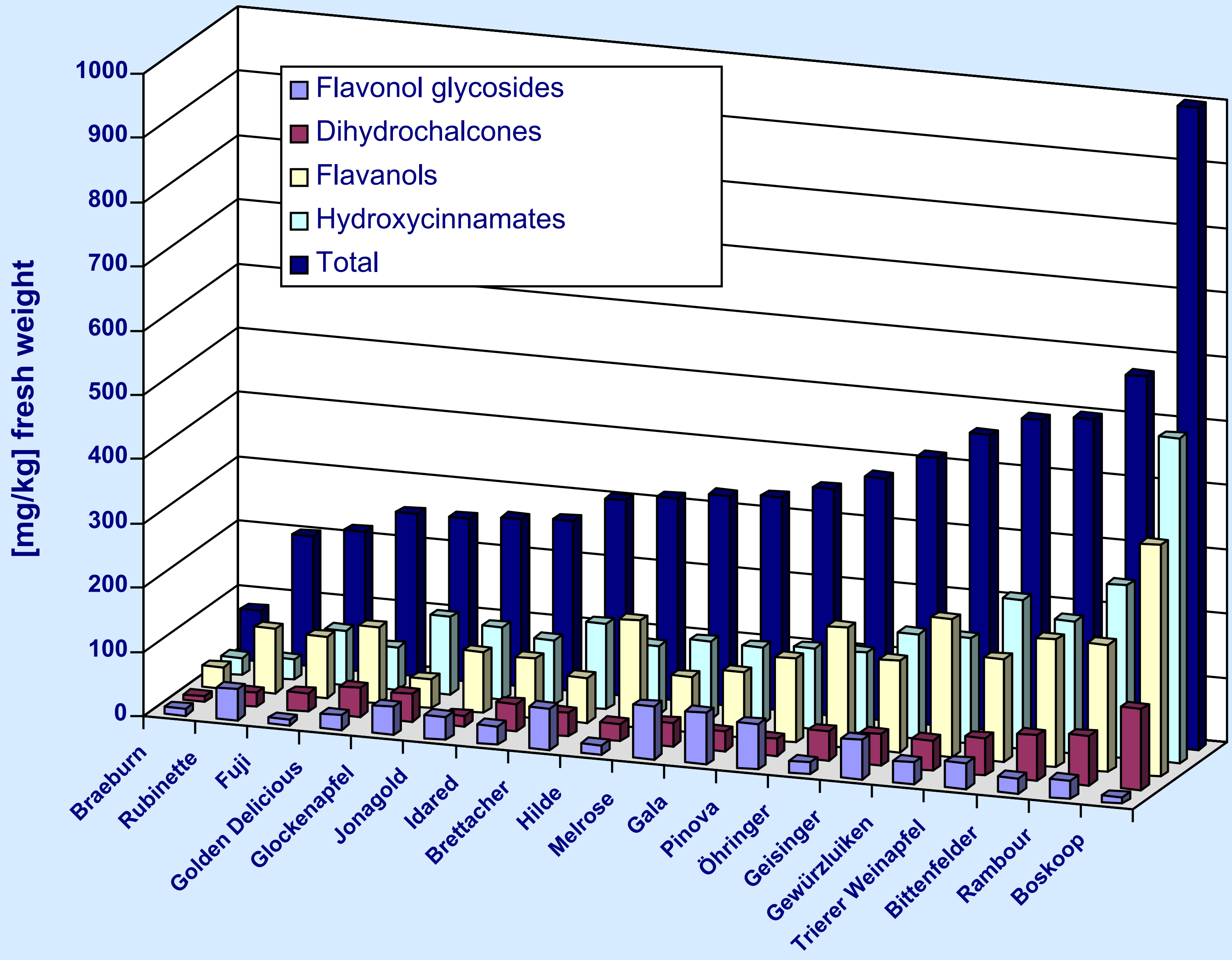
[1] Drying by lyophilisation

[2] Drying in a drum dryer (8 min, 300 - 700 °C)

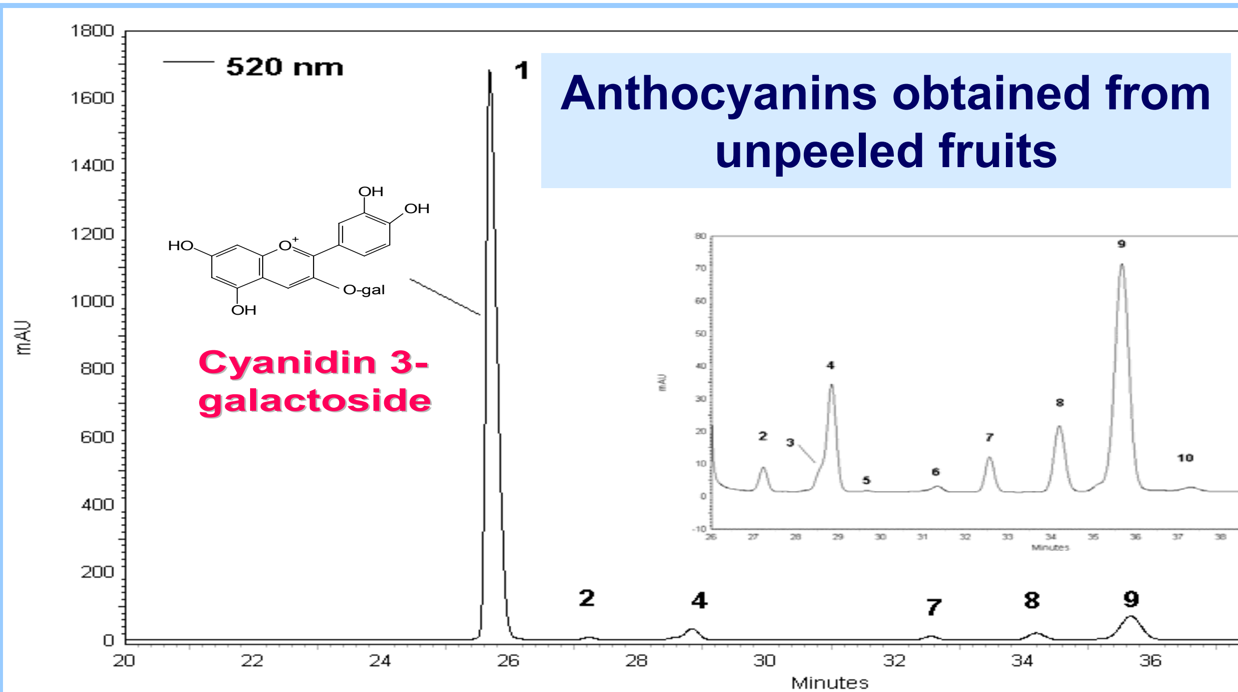
Schieber, A., Keller, P., Carle, R. (2001) Journal of Chromatography A 910, 265-273

POLYPHENOLIC CONTENTS IN APPLES

(cider and table apple varieties)



Keller, P., Streker, P., Arnold, G., Schieber, A., Carle, R.
Flüssiges Obst 68, 480-483 (2001)



Anthocyanins obtained from unpeeled fruits

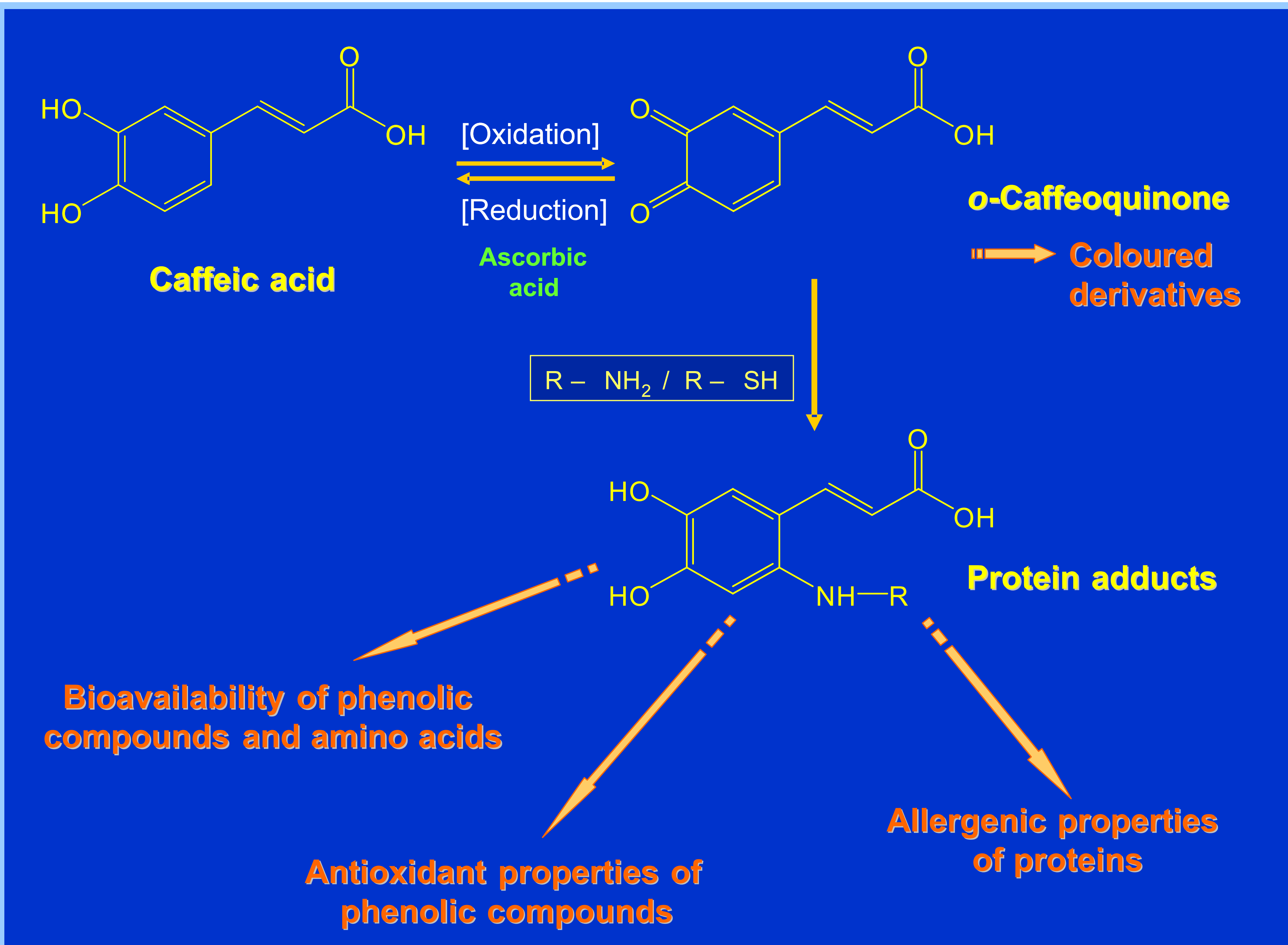
‚Weirouge' apples

Antioxidant capacity [mg/kg] of edible part fractions

Parameter	unpeeled apple	apple flesh	apple peel
TEAC [Trolox-equivalent]	1463 ± 179	911 ± 83	4879 ± 522
FRAP [Trolox-equivalent]	599 ± 63	374 ± 82	1772 ± 237
TEAC [Vitamin C-equivalent]	888 ± 109	563 ± 51	3004 ± 317
FRAP [Vitamin C-equivalent]	403 ± 39	243 ± 56	1166 ± 170
Total phenolics [Gallic acid-equivalent]	538 ± 42 [b]	379 ± 22	1684 ± 184
Total phenolics [Vitamin C-equivalent]	784 ± 59 [b]	540 ± 28	2385 ± 248

[a] mean value ± standard deviation
[b] corrected by the ascorbic acid content of 22 mg

Sadilova et al., J. Appl. Bot. Food Qual. 80, 82-87 (2006)

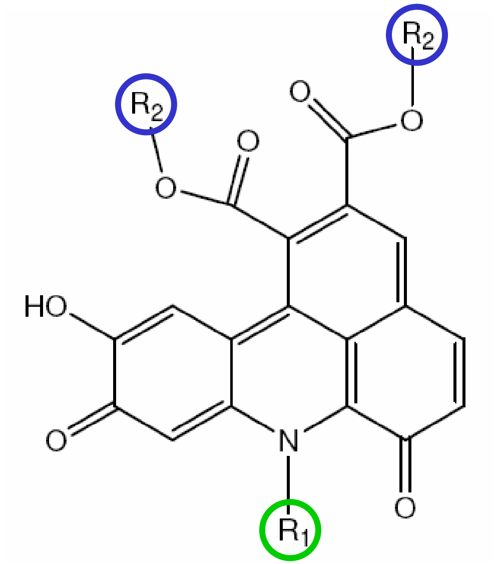


‘The role of chlorogenic acid and other polyphenols during food processing is largely unknown.‘

Mendel Friedman (1997)

Covalent addition products of chlorogenic acid quinone (CQA) with amino acid derivatives

According to Namiki et al. (2001)

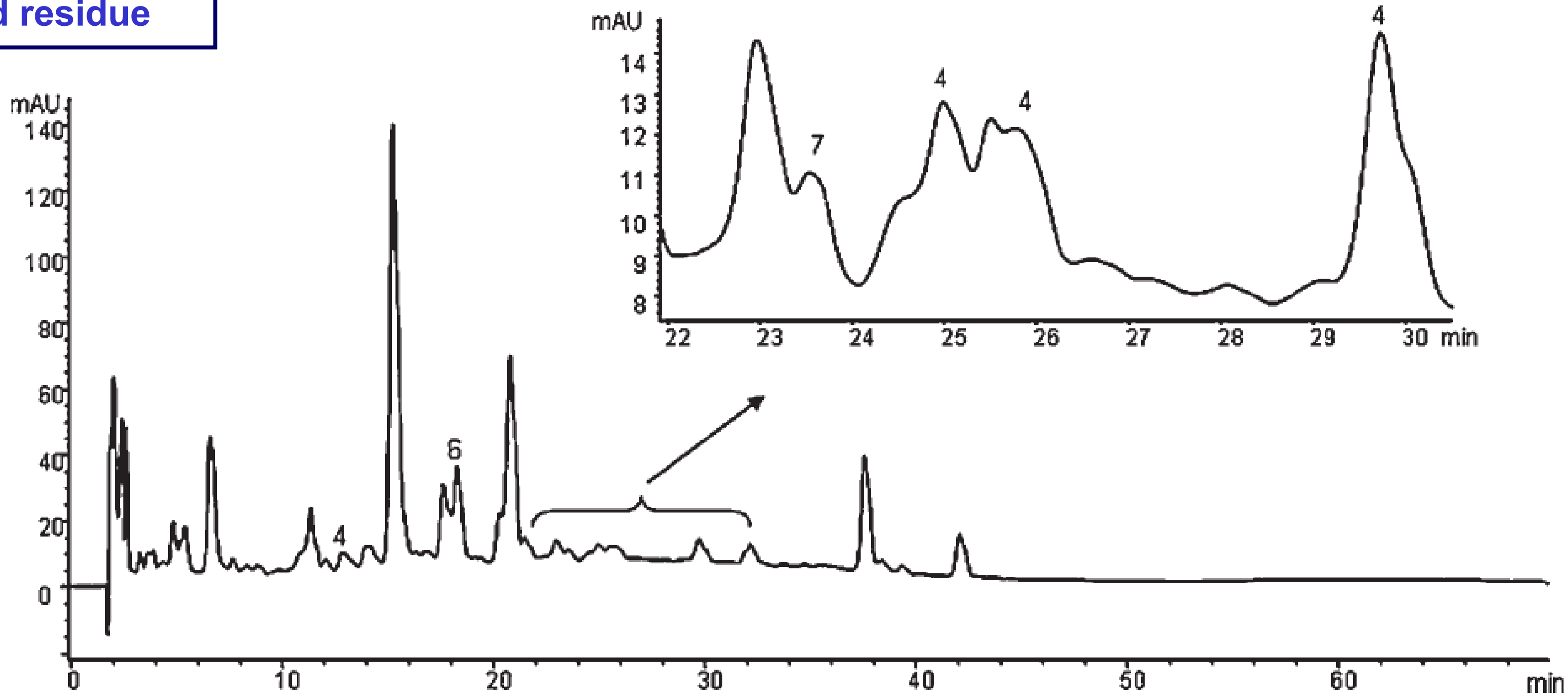


R_1: Quinic acid residue

R_2: Amino acid residue

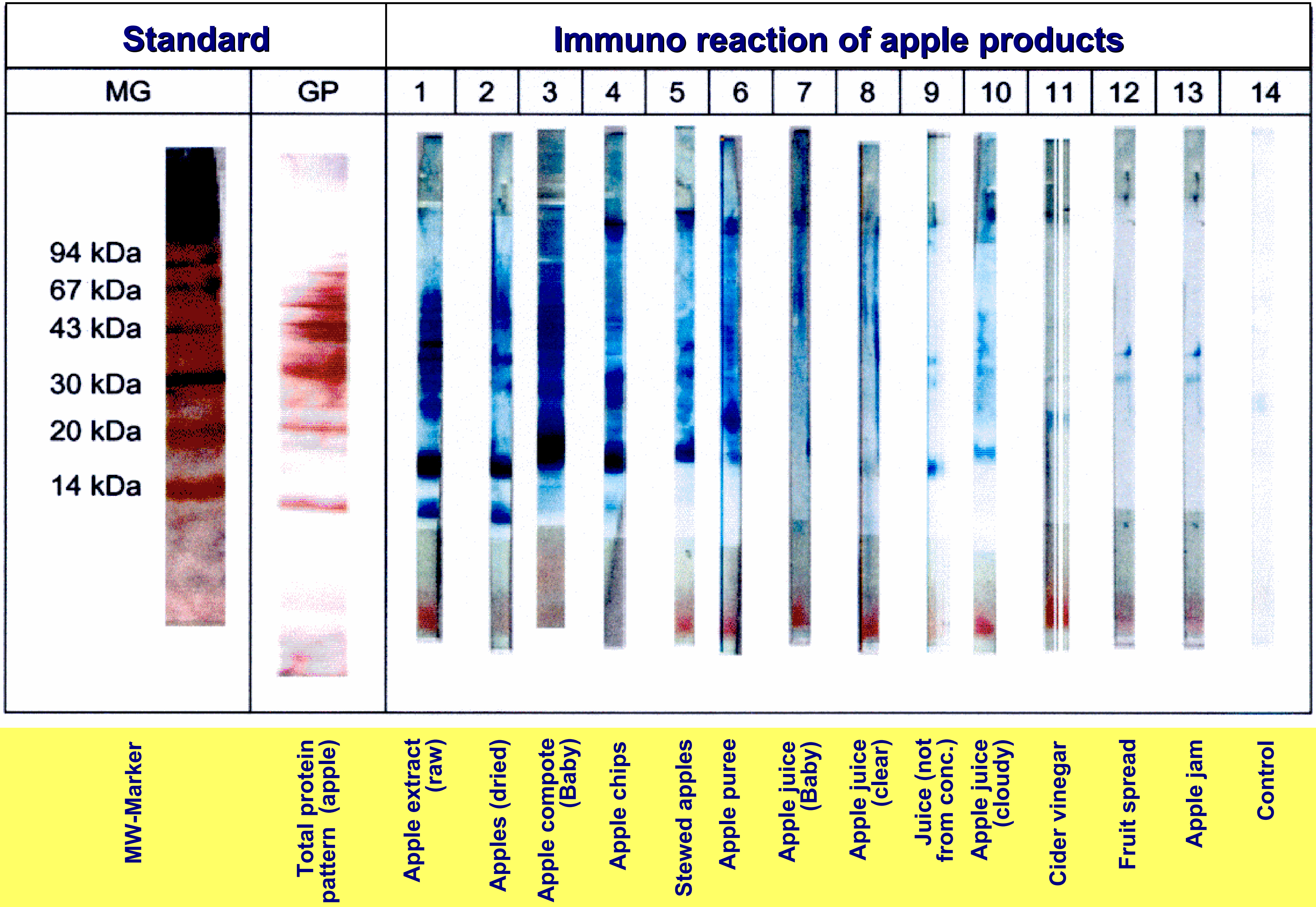
Apple juice (pH 3.6) with added *N*-acetyl-L-cysteine (*N*-Ac-Cys) (separation by HPLC, 280 nm)

4: Dimer of CQA
5: Monoaddition product (N-Ac-Cys + CQA)
7: Diaddition product (2 N-Ac-Cys + CQA)

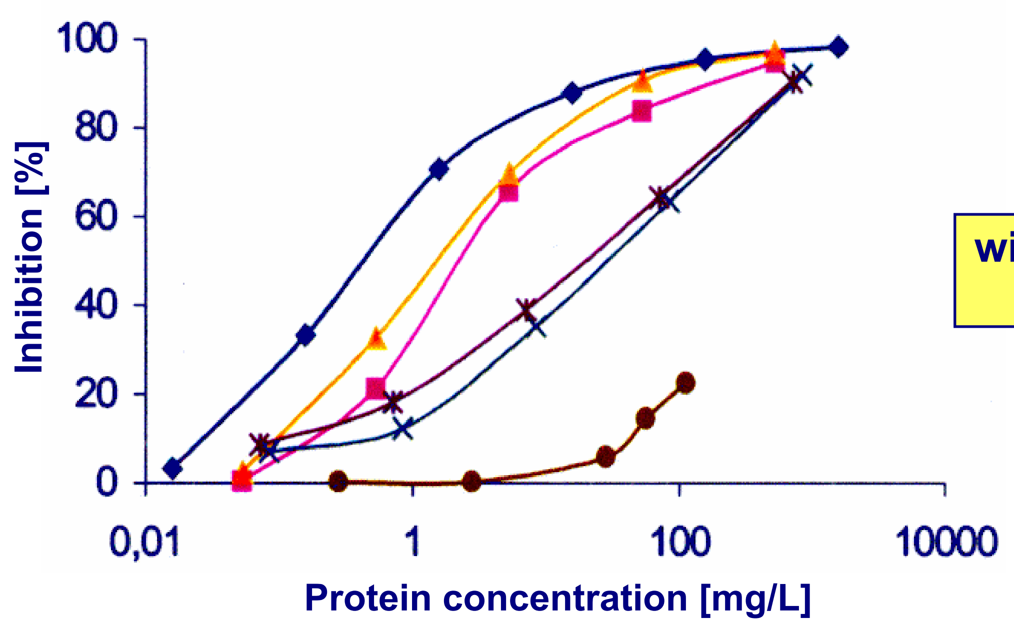


Schilling, S., Sigolotto, C.-I., Carle, R., Schieber, A., Rapid Commun. Mass Spectrom. 22, 441-448 (2008)

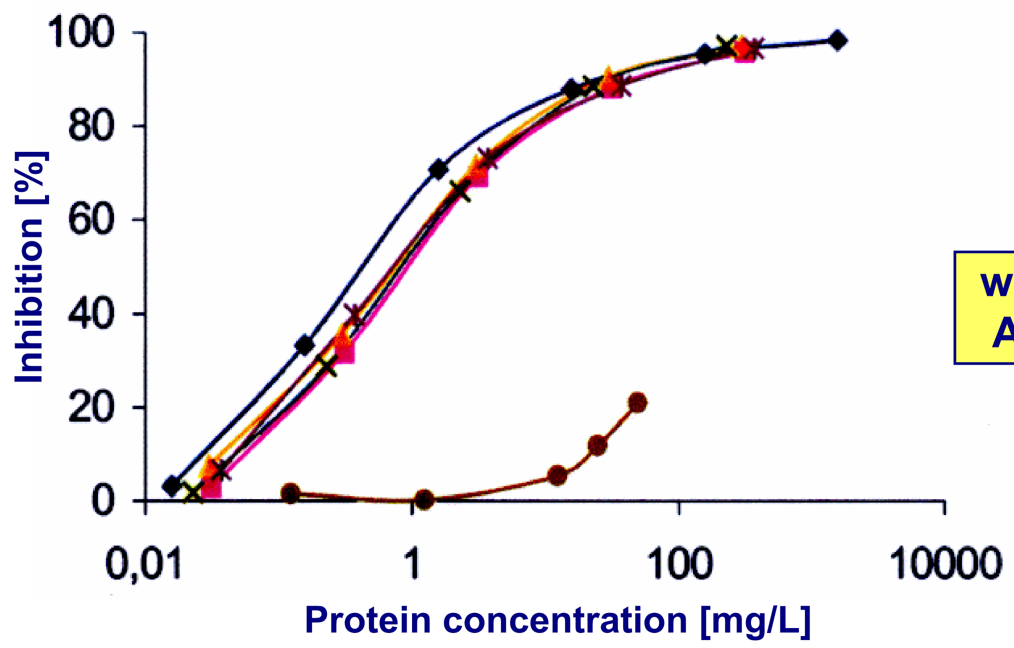
IMMUNOBLOT OF COMMERCIAL APPLE PRODUCTS



EAST inhibition of apple mashes without and with ascorbic acid (AA)



without AA



with AA

Golden Delicious

- Apple raw
- Mash 0 h
- Mash 1 h
- Mash 2 h
- Mash 3 h
- Finished product (pasteurised)

C_{50} values [mg/L]

		without AA	with AA
Apple raw		0,45	0,45
Mash	0 h	2,4	0,99
	1 h	1,6	0,77
	2 h	29	0,87
	3 h	20	0,77
Finished product		-	-

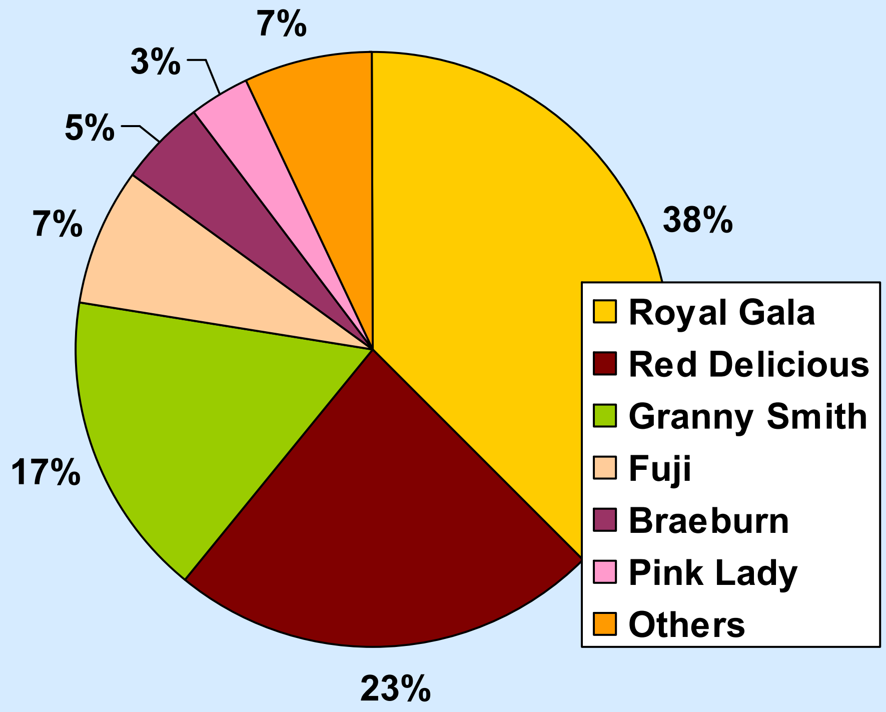
Hoppe, S., Wigotzki, M., Zunker, K., Neidhart, S., Carle, R., Steinhart, H., Paschke, A., Lebensmittelchemie 57, 155 (2003)

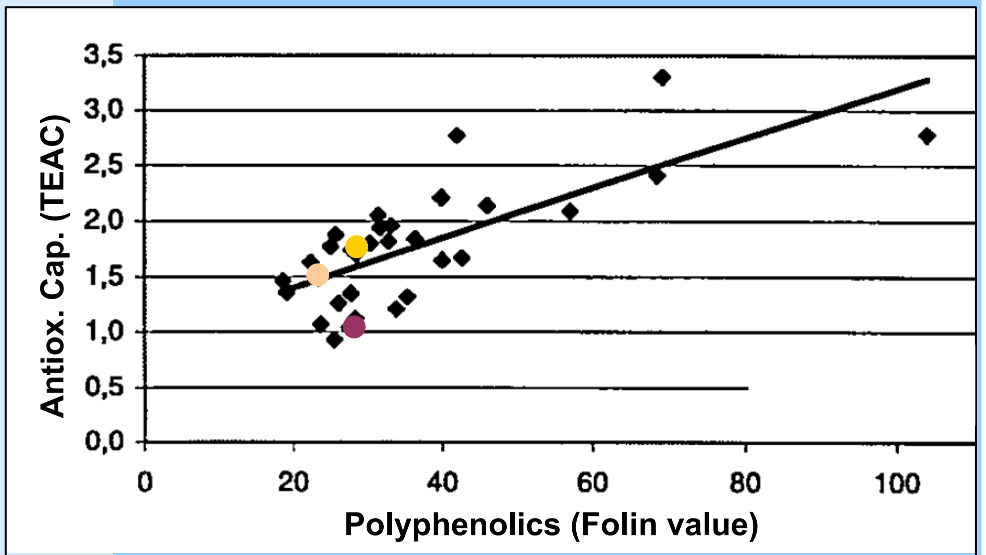
Traditional apple varieties are more suitable for allergic persons

20 % of the population in Western Europe suffer from incompatibilities caused by foodstuffs (especially from fruits) → increasing tendency

Newly-breeded apple varieties generate considerably more allergies than traditional varieties. Especially Golden Delicious is highly allergenic!

Apple varieties produced in Chile





FUNCTIONALITY OF APPLE COMPONENTS

Techno-functionality

Technological properties

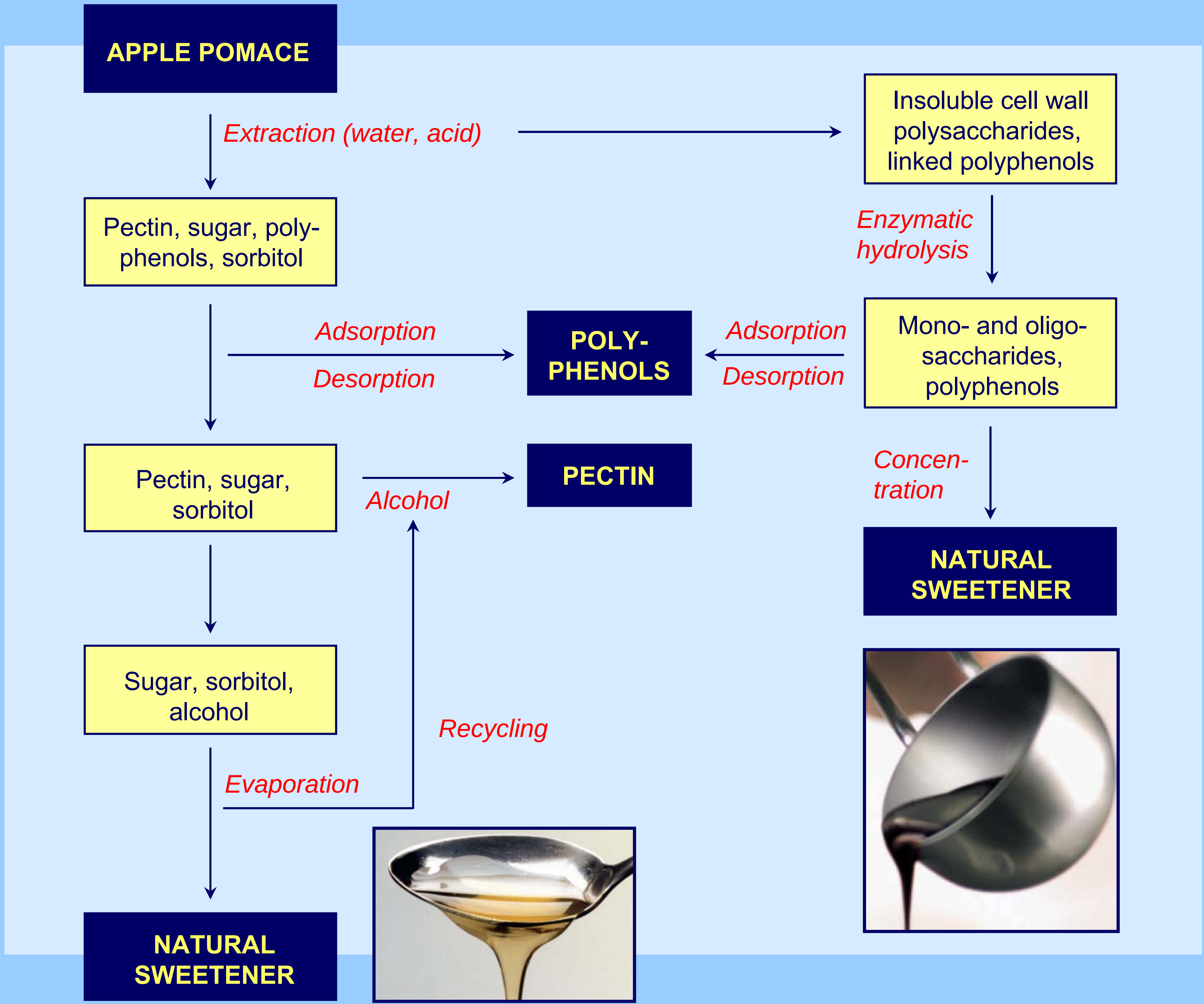
- Texture (gelling agent)
- Antioxidant
- Sweetener

Bio-functionality

“Added value” beyond the nutritional value

- Pre-/Probiotics
- Antioxidant

RECOVERY OF VALUABLE SUBSTANCES FROM APPLE POMACE



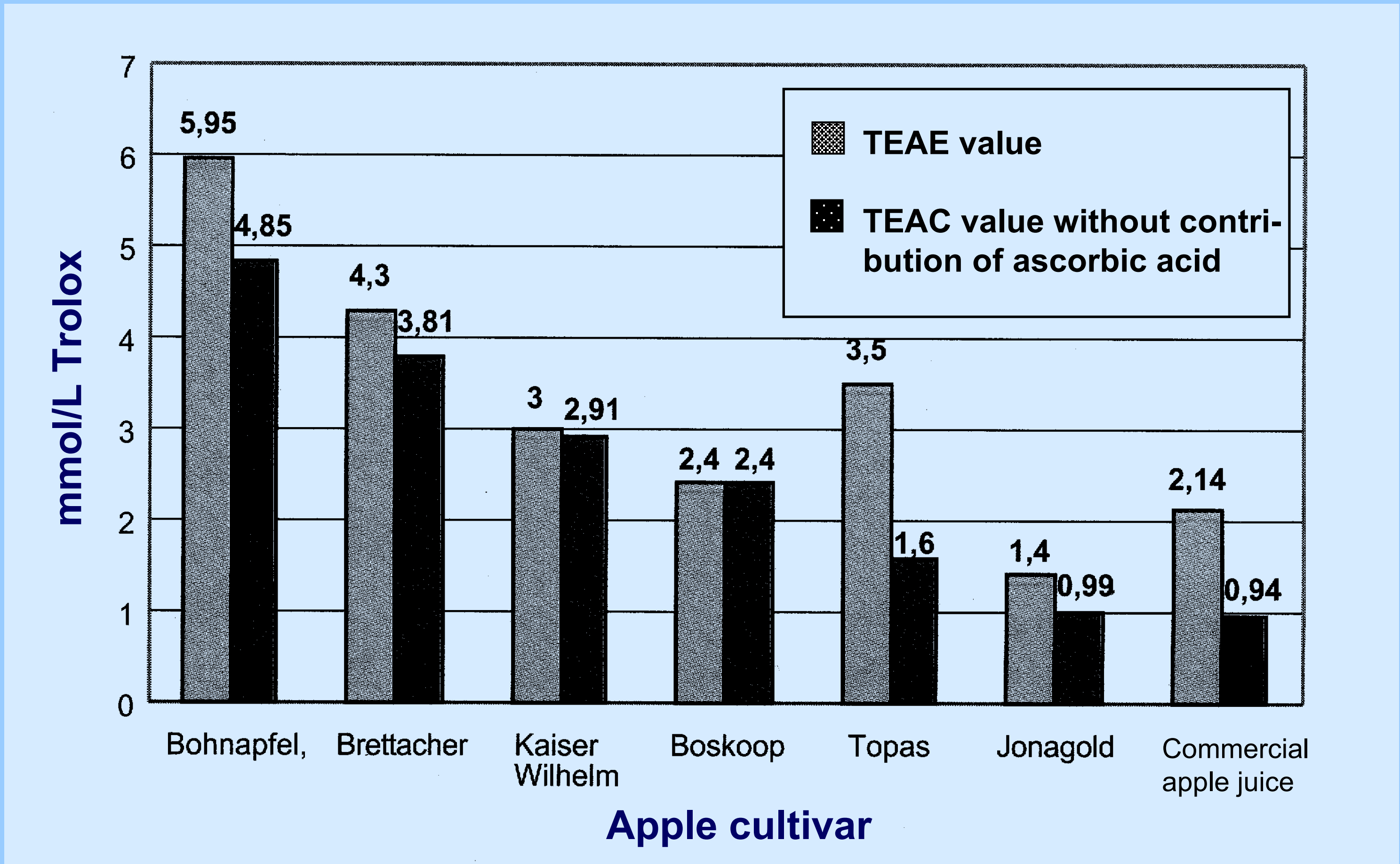
PHYSIOLOGICAL EFFECTS OF APPLE POLYPHENOLS

(selection)

- **Antioxidative effect**
  - Radical scavenger
- **Protection of blood vessels and cardiovascular system**
  - Lipid oxidation
  - Antithrombotic effect
- **Cancer prevention**
- **Additional effects**
  - Antiviral
  - Anticariogenic
  - Antiinflammatory
  - UV protection

Impact of cultivar on the antioxidant capacity of cloudy apple juices

(TEAC: Trolox Equivalent Antioxidative Capacity)



Rechner, A. et al.: Flüss. Obst 66, 227-230 (1999)

For comparison:

Black current and sour cherry nectars (fruit content 25 and 40 %, resp.): TEAC values > 7,5 mMol/L

Application of an apple pomace extract as a natural antioxidant in pizza salami

Storage of deep-frozen pizza in the presence of oxygen

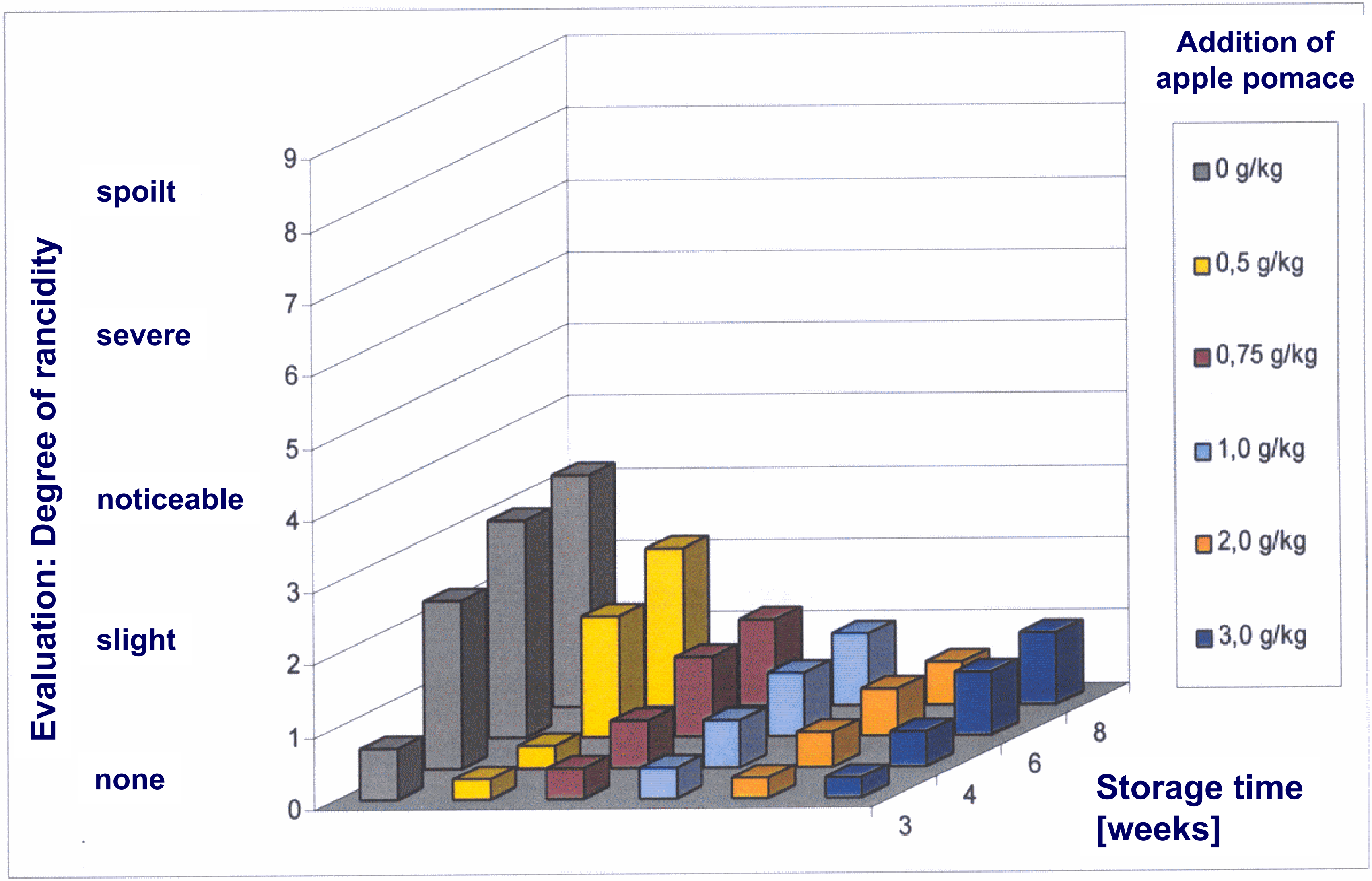
↓ **Limitation:** **Lipid oxidation**

Shelf-life of raw sausage

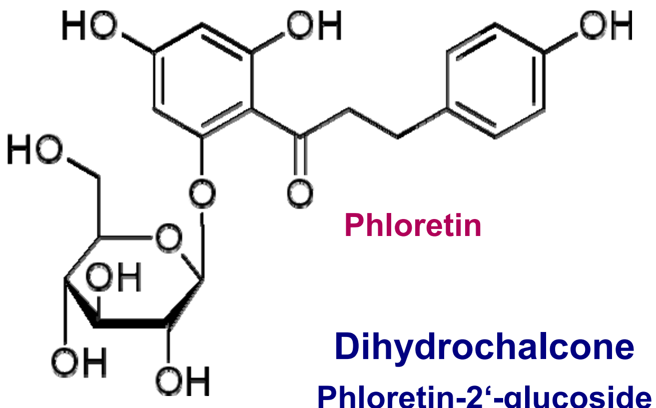
Addition of apple pomace extract:

→ Inhibition of lipid oxidation depending on the quantity added

Sensory evaluation of pizza salami depending on the addition of apple pomace extract and the storage time at -18 °C



Arelt, A., Hilt, P., Kirsch, P., Schieber, A., Carle, R., Fischer, A. (2002) Symposium der Lebensmittelchemischen Gesellschaft, 83-84



Dihydrochalcone

Phloretin-2'-glucoside

bitter

Phlorizin

Contents in apples

- Peels	12 - 418 mg/kg fresh weight
- Flesh	4 - 20 mg/kg fresh weight
- Seeds	80 % of total phenolics (760 mg/kg dry matter)

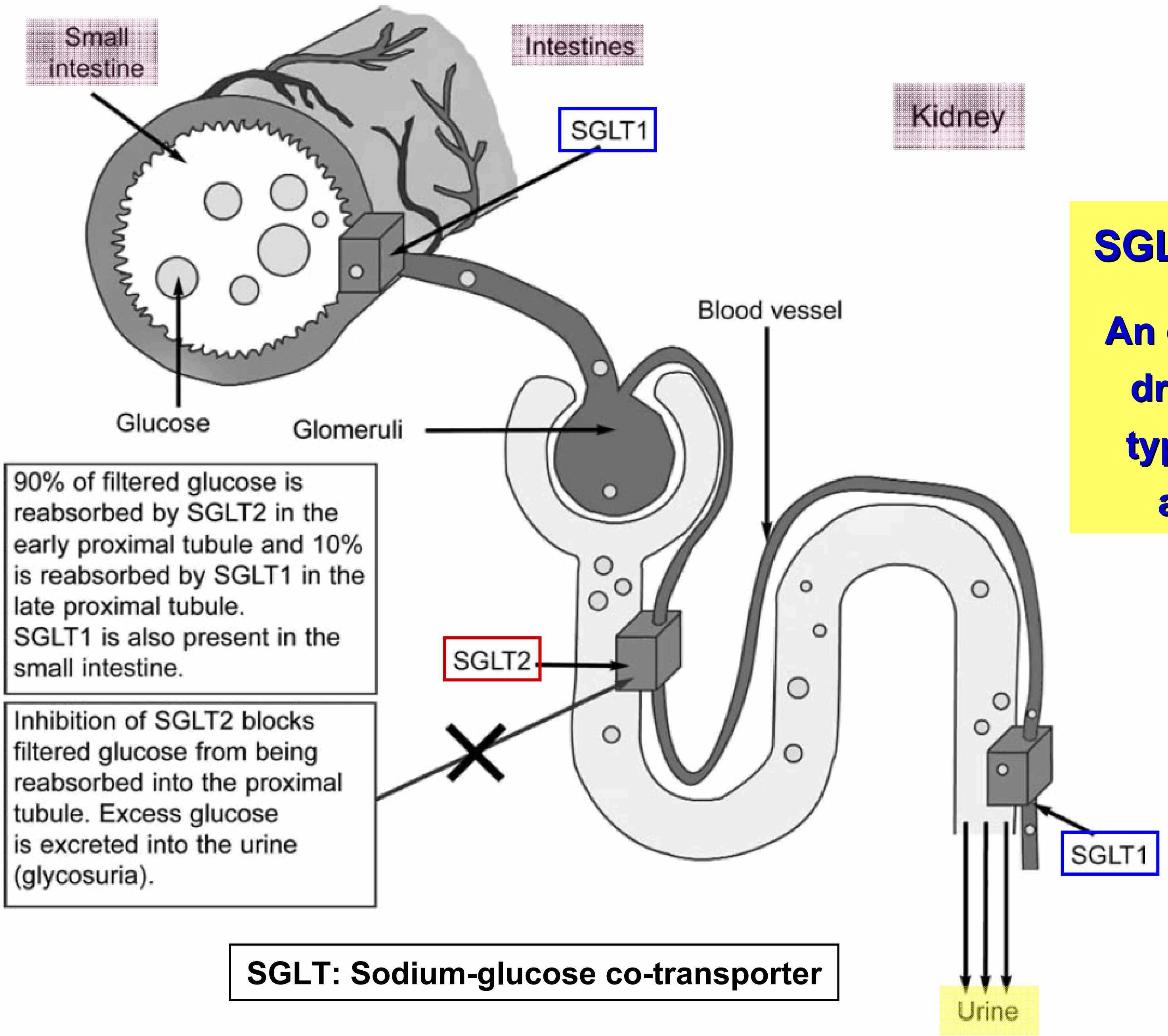
History

1835	French chemists isolated phlorizin from the bark of the apple tree *greek: phloos* bark, *rhiza* root Due to the bitter taste: Assumption of antipyretic properties similar to that of willow and cinchona extracts → treatment of fevers and malaria
1886	*von Mering*: Doses greater than 1 g produce glucosuria
1887	*Merck Index*: „Glycosid aus der Wurzelrinde des Apfelbaums“
1903	Tool for the study of renal function in humans
1930	Intravenous administration of phlorizin Noninvasive clearance method to measure glomerular filtration rate and renal blood flow
1970	Treatment of malignant diseases by attempting to block glucose uptake by tumor cells

Ehrenkranz, J.R.L. et al., Diabetes Metab. Res. Rev. 21, 31-38 (2005)

SGLT2 inhibitors

An emerging new drug for use in type 2 diabetes and obesity

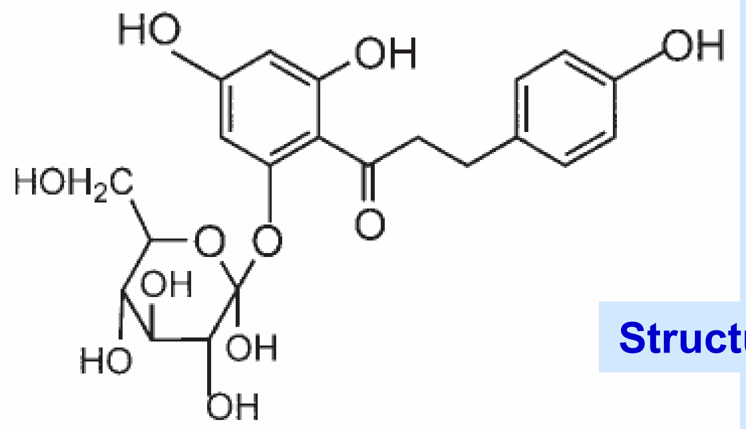


Idris, I., R. Donnelly, Diabetes, Obesity and Metabolism, 11, 79-88 (2009)

SGLT2 inhibitors derived from phlorizin

Phlorizin

- \+ Lowers blood glucose without insulin secretion
- \- Nonselective SGLT inhibitor
- \- Bioavailability (hydrolyzation)
- \- Phloretin (aglycon): Inhibition of glucose transporter GLUT1

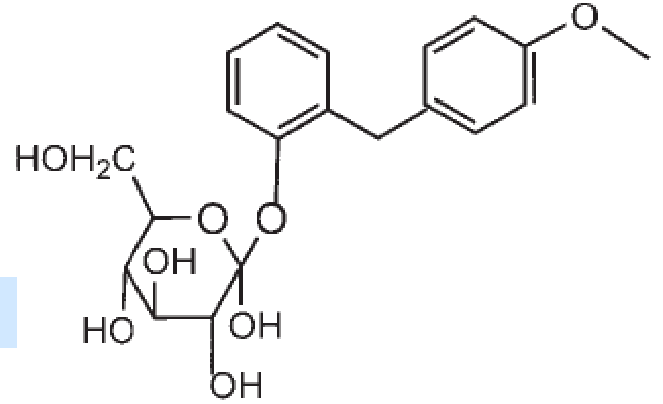


Sergliflozin-A

Compared with phlorizin:

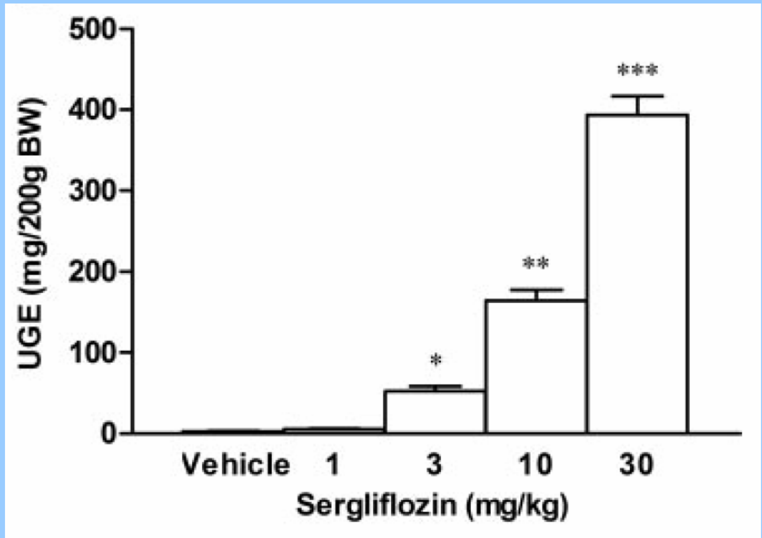
7 times more active against SGLT2

Only one-fifth as active against SGLT1



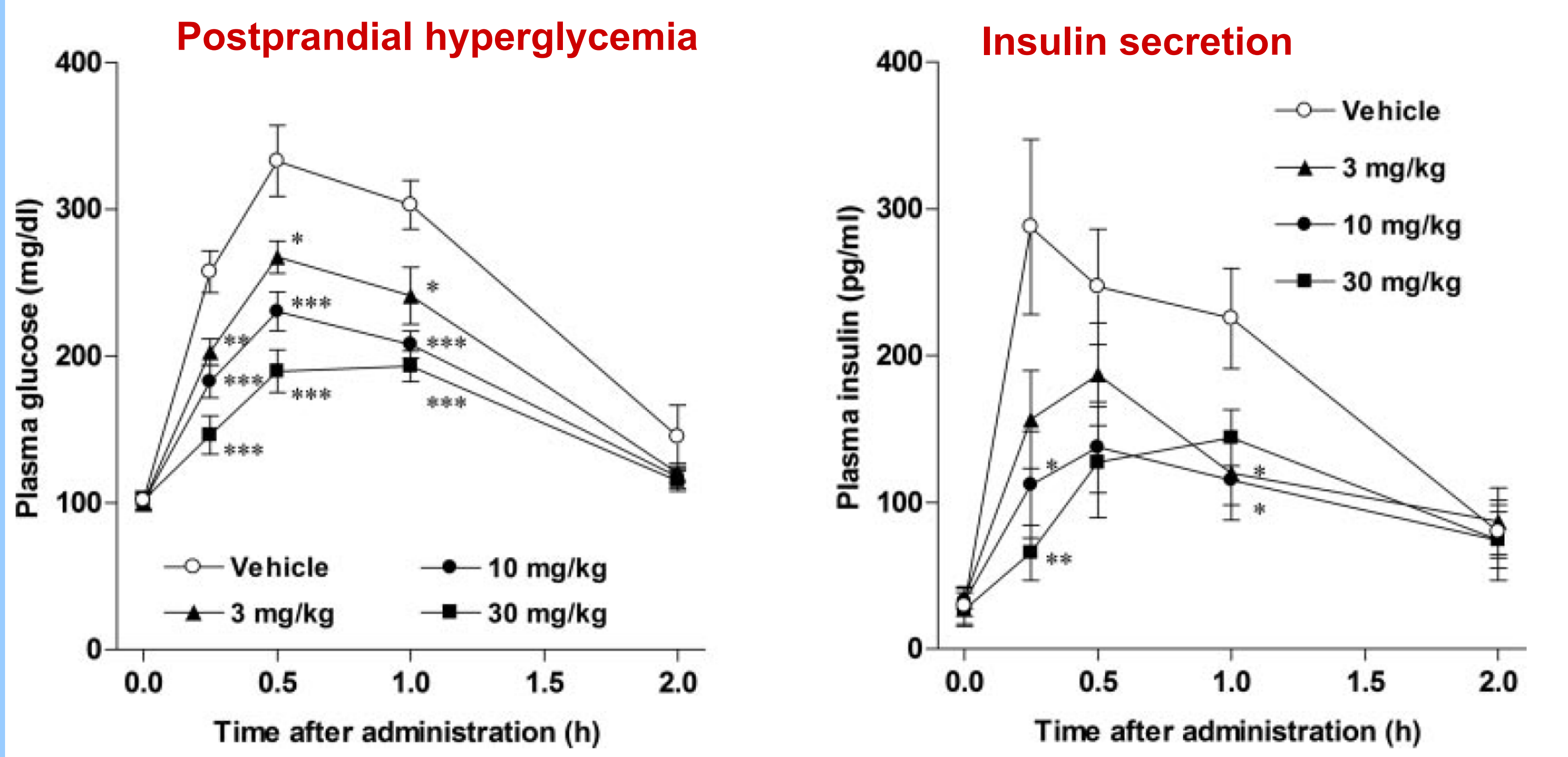
Structurally related

Katsuno, K. et al., The Journal of Pharmacology and Experimental Therapeutics, 320, 323-330 (2007)



Sergliflozin increases urinary glucose excretion (UGE) in rats

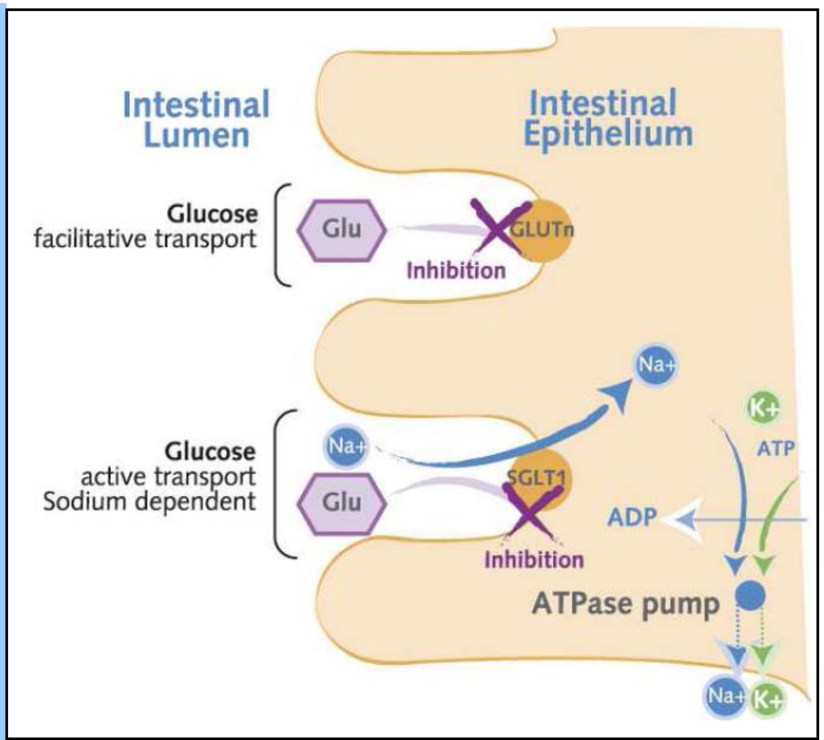
Effects of sergliflozin in oral glucose tolerance test in diabetic rats

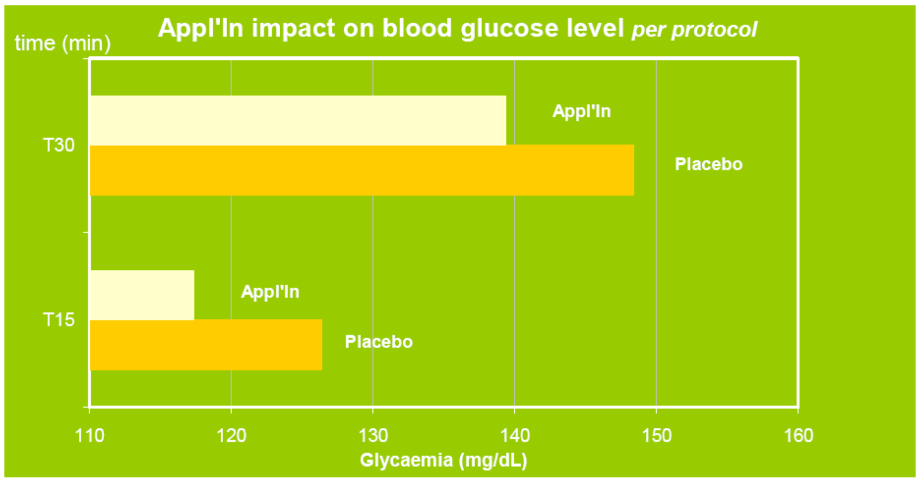


A Natural Ingredient for Weight Management

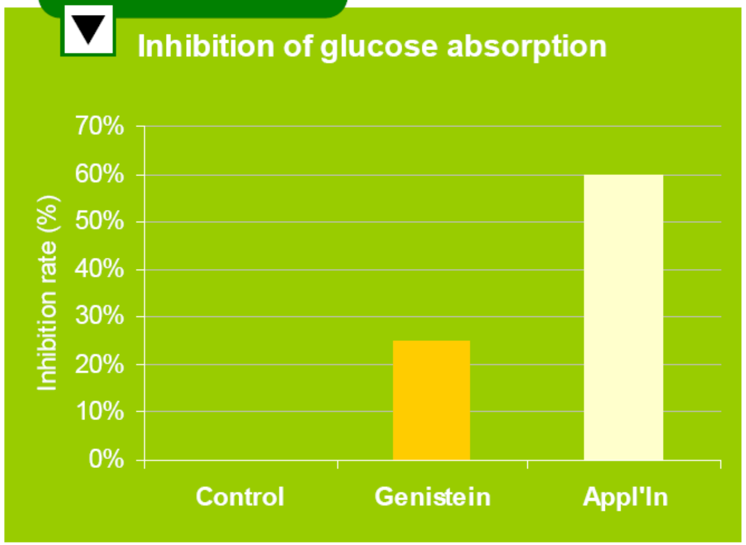
Appl'in™ by Phytonutriance®

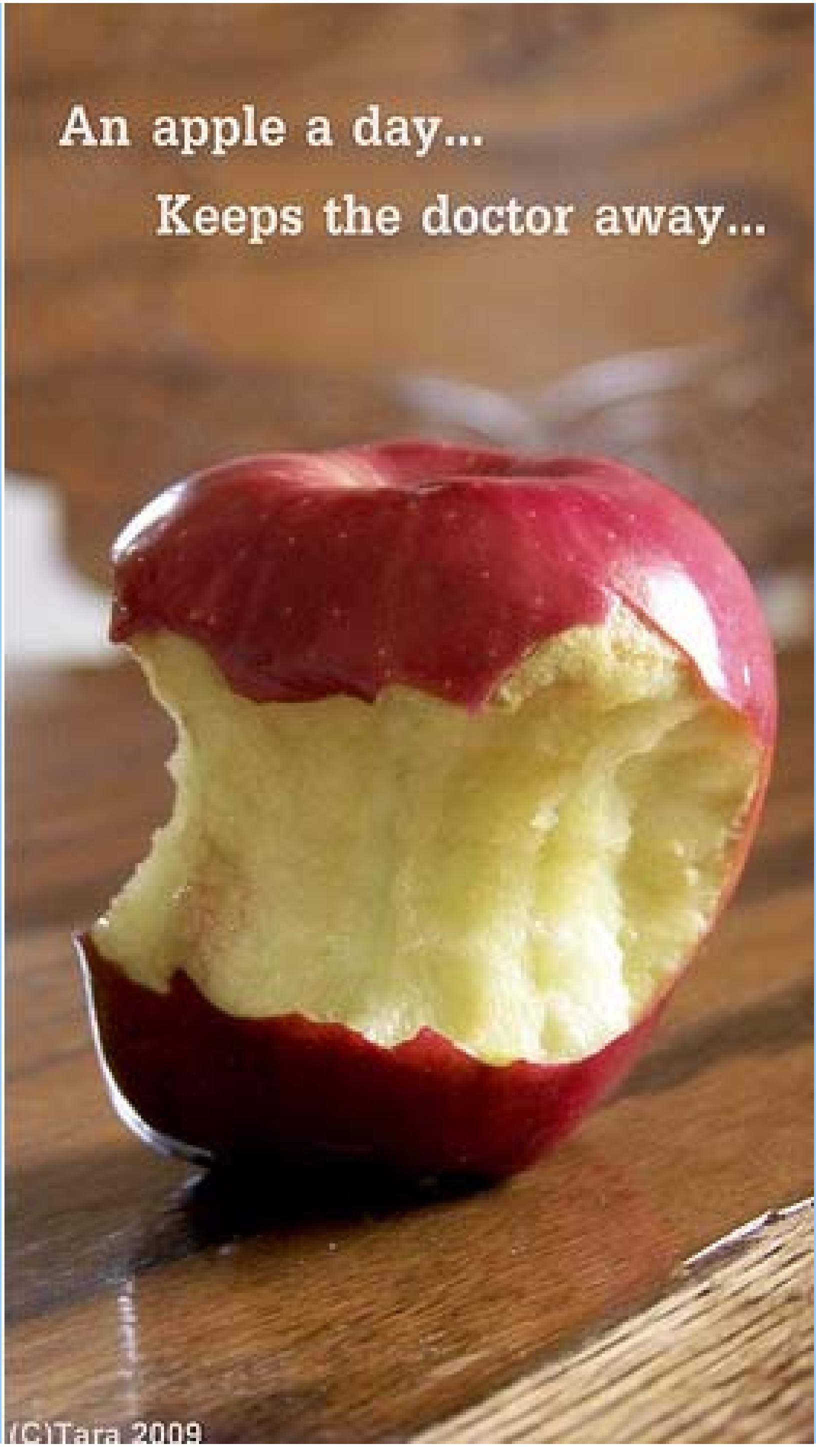
> Apple extract powder
> Standardized to 80% total polyphenols, 5% phloridzin
> Glucose blocker
> Patented product supported by human clinicals
> May promote satiety effect





Appl'In™ inhibits almost 60% of the glucose absorption





Eat an apple on going to bed, and you'll keep the doctor from earning his bread.

Welsh Journal, 1866


Thank you
for your attention!