Fermentation
How does it effect the polyphenols?

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Amsterdam – 23 May 2007

Topics

- What are polyphenols?
- Communication of polyphenols on commercial chocolate
- Health aspect polyphenols
- Impact cocoa process on polyphenols
- How maintaining high polyphenol levels

Cocoa: natural, untapped potential

Acetic-acid, aesculetin, alanine, alkaloids, alpha-sitosterol, alpha-theosterol, amyl-acetate, amyl-alcohol, amyl-butyrate, amylase, apigenin-7-o-glucoside, arabinose, arachidic-acid, arginine, ascorbic-acid, ascorbic-acid-oxidase, aspariginase, **beta-carotene**, **beta-sitosterol**, beta-theosterol, biotin, caffeic-acid, **caffeine**, calcium, campesterol, catalase, **catechins**, catechol, cellulase, cellulose, chlorogenic-acid, chrysoeriol-7-o-glucoside, citric-acid, coumarin, cyanidin, cyanidin-3-beta-l-arabinoside, cyanidin-3-galactoside, cyanidin-glycoside, cycloartanol, d-galactose, decarboxylase, dextrinase, diacetyl, **dopamine**, **epicatechin**, ergosterol, ferulic-acid, formic-acid, fructose, furfurol, galacturonic-acid, gallocatechin, gentisic-acid, glucose, glutamic-acid, glycerin, glycerophosphatase, glycine, glycolic-acid, glycosidase, haematin, histidine, i-butyric-acid, idaein, invertase, isobutylacetate, isoleucine, isopropyl-acetate, isovitexin, kaempferol, **l-epicatechin,** leucine, leucocyanidins, linalool, linoleic-acid, lipase, luteolin, luteolin-7-o-glucoside, lysine, lysophosphatidyl-choline, maleic-acid, mannan, manninotriose, mannose, melibiose, mesoinositol, methylheptenone, n-butylacetate, n-nonacosane, **niacin**, nicotinamide, nicotinic- acid, nitrogen, nonanoic-acid, o-hydroxyphenylacetic-acid, octoic-acid, **oleic- acid**, oleo-dipalmatin, oleopalmitostearin, oxalic-acid, p-anisic-acid, p-coumaric-acid, p-coumarylquinic-acid, p-hydroxybenzoic-acid, p-hydroxyphenylacetic-acid, palmitic-acid, palmitodiolen, pantothenic-acid, pectin, **pentose**, peroxidase, phenylacetic-acid, phenylalanine, phlobaphene, phosphatidyl-choline, phosphatidyl- ethanolamine, phosphatidyl-inositol, **phospholipids,** phosphorus, phytase, planteose, polygalacturonate, polyphenol-oxidase, **polyphenols, proline**, propionic-acid, propyl-acetate, protocatechuic-acid, **purine**, pyridoxine, **quercetin**, quercetin-3-o-galactoside, quercetin-3-o-glucoside, quercitrin, raffinase, raffinose, reductase, rhamnose, riboflavin, rutin, rutoside, saccharose, salsolinol, serine, sinapic-acid, stachyose, stearic-acid, stearodiolein, **stigmasterol**, sucrose, syringic-acid, **tannins,** tartaric-acid, **theobromine**, **theophylline**, **thiamin**, threonine, trigonelline, tyramine, tyrosine, valerianic-acid, valine, vanillic-acid, verbascose, verbascotetrose, vitexin

Cocoa contains hundreds of different components of which many have potential health benefits.

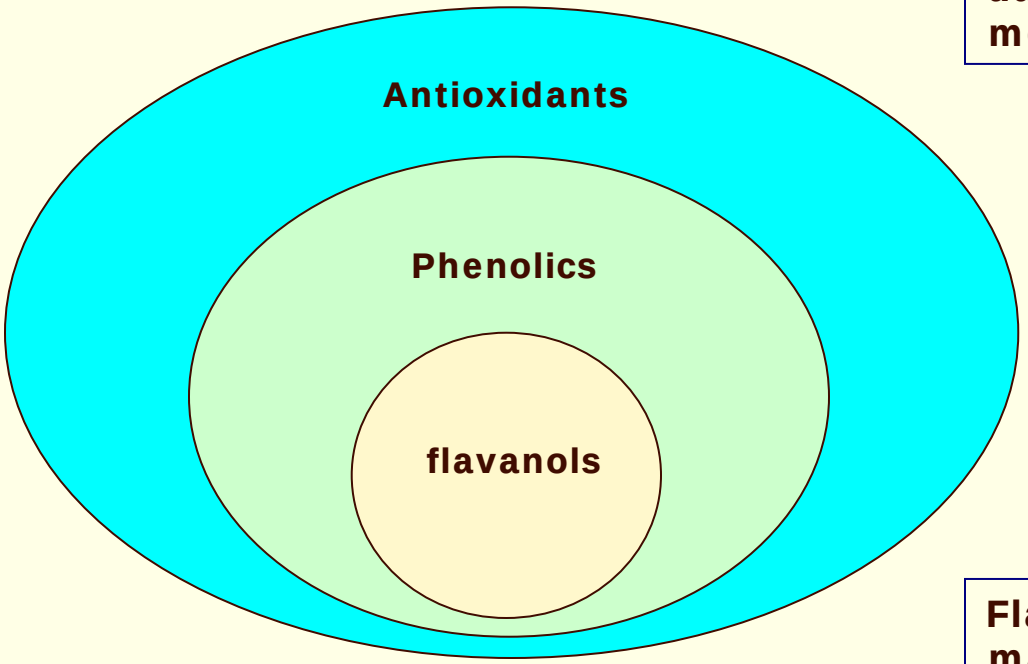
New products bearing health positioning claims, 2002-2004				
"Foods Plus" positioning claims	2002	2003	2004	**Total**
Vitamin/Mineral Fortified	4,599	4,846	5,808	**15,253**
All Natural	2,238	2,992	4,108	**9,338**
Organic	2,663	2,682	2,748	**8,093**
Added Calcium	1,315	1,233	1,344	**3,892**
Functional	941	889	1,237	**3,067**
Added Fiber	690	678	665	**2,033**
Diabetic	148	302	628	**1,078**
Low/No/Reduced Glycemic	21	46	105	**172**
Source: Mintel's Global New Products Database				

"Functional foods increase, but no real functional chocolate"

"What about polyphenols and the French paradox"

What are polyphenols?

Relation between anti-oxidants and polyphenols



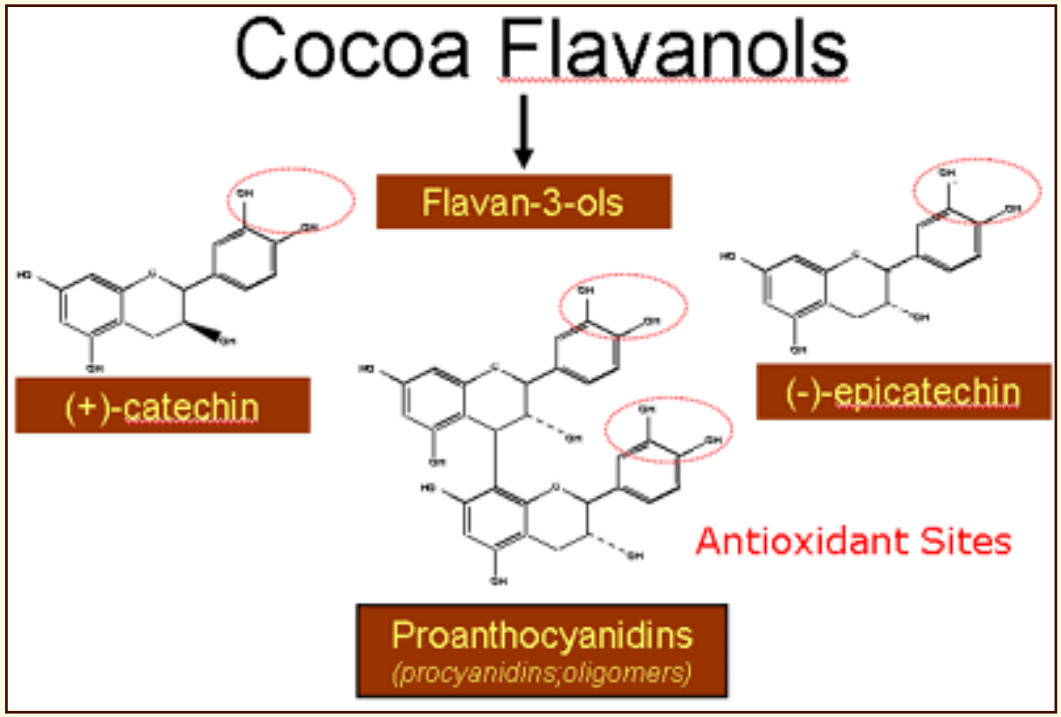
Antioxidant - a chemical that hinders oxidative damage (e.g. vitamins C and E), ORAC is one measurement for antioxidant power Value

Phenolics - Class of naturally occurring compounds that behave as antioxidants

Flavanols are a specific category of phenolics mainly found in cocoa and are generally measured using LC - methodology

Cocoa polyphenols

Background: Polyphenols are plant components



Polyphenols (10) => flavonoids (13) => flavanols

Polyphenols are stored in the cotyledon pigment cells

Cocoa and chocolate Flavonoids.

- (-)-Epicatechin
- (+)-Catechin

} = flavan-3-ols (37%)

- Anthocyanins (4%)
- Proanthocyanidins (58%)

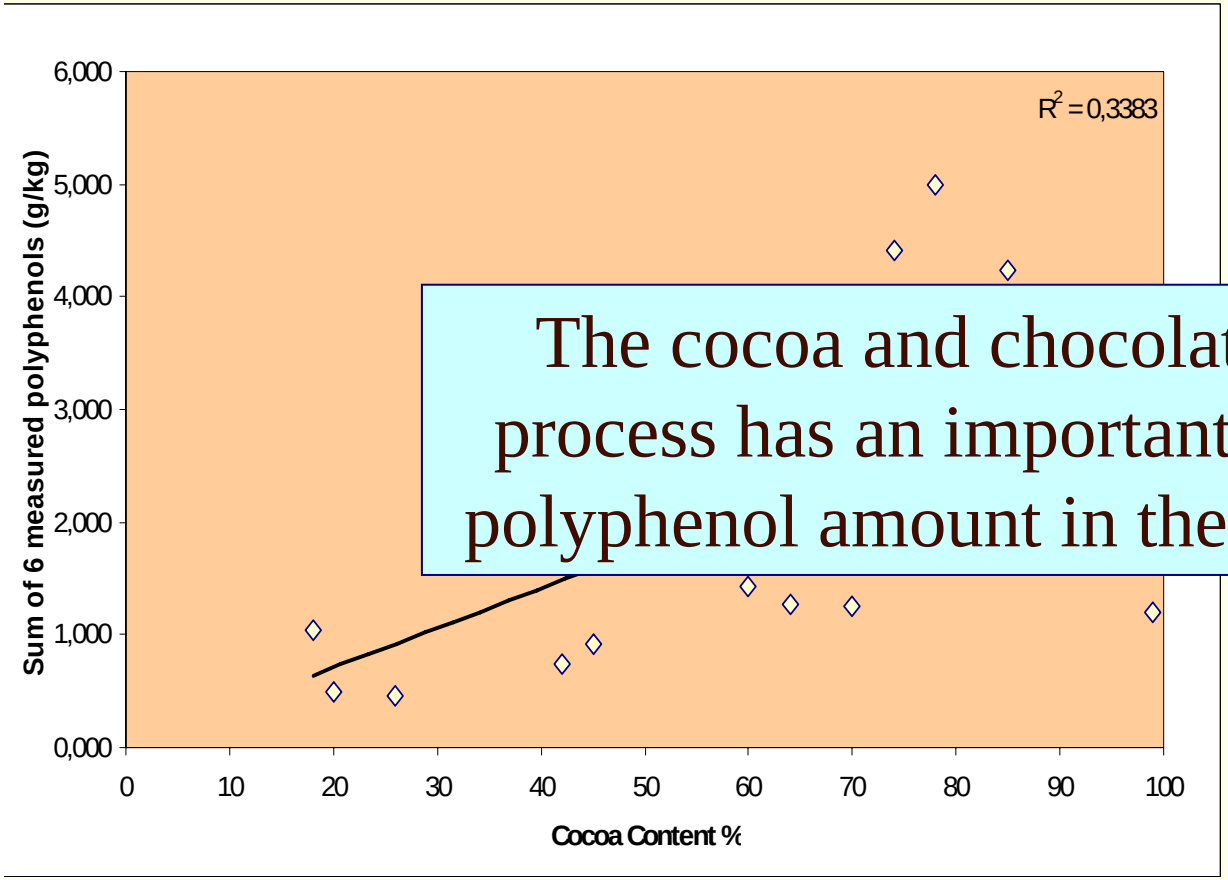
Cocoa contains more polyphenols then red wine or tea. Additionally cocoa polyphenols are more active as an anti-oxidant

Forastero > Criollo (2/3 polyphenols)

mg flavan-3-ol / 100g or 100ml
Apple 5-15
Chocolate 20-400
Red Wine 2.2-45
Black Tea 7.5-35

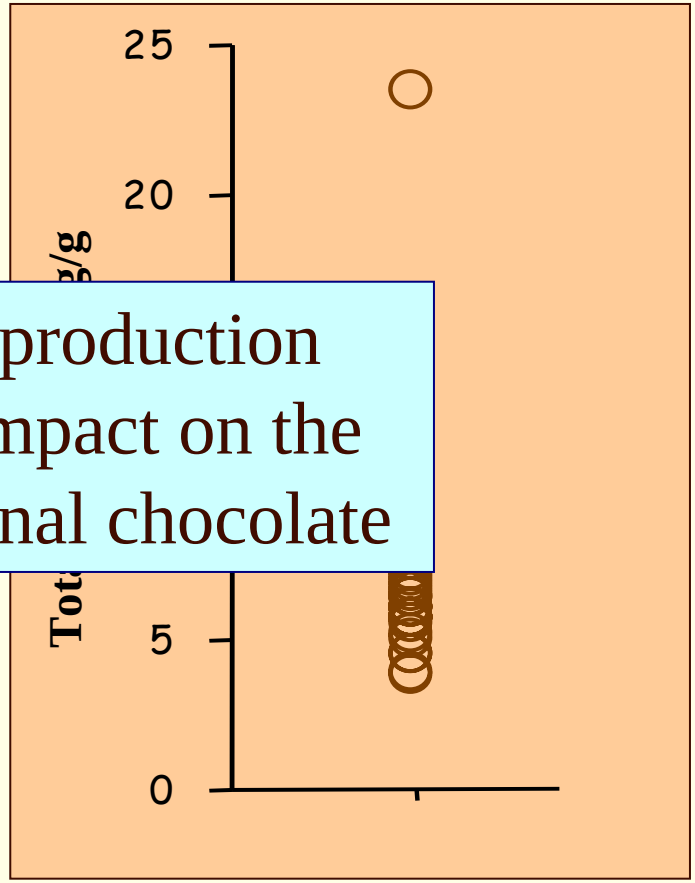
Chocolate and polyphenols

Relationships between labelled cocoa content and sum of 6 measured polyphenols in 32 chocolate samples



Procyanidin profiles of cocoa-rich products by ultra performance liquid chromatography (Gimenez et al, 2006,) – 2nd International Conference on Polyphenols and Health, University of California, Davis

Amount of total measured phenolic content in 34 commercial chocolate samples



Roger Corder, 2006, William Harvey Institute

The cocoa and chocolate production process has an important impact on the polyphenol amount in the final chocolate

Cocoa polyphenols

Why keeping them in the bean?

Focus on health – compelling claim areas

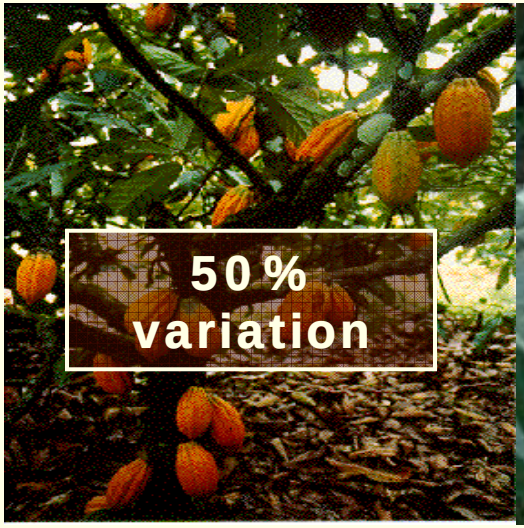
- **Cardiovascular health**
  - Reduced blood pressure
  - Platelet function / endothelium function
  - Lower bad cholesterol (oxidised LDL cholesterol)
- **Cognitive performance**
  - Improved blood flow
  - Cognitive studies on animals and humans
  - Cognitive studies other polyphenol sources
  - Indirect effect via environment – antioxidant effect
- **Anti-aging**
  - Link to cardiovascular health
  - Link to cognitive performance and activity
  - Oxidative stress
- **Immune response**
  - Inflammation
  - Effect on leucocytes and cytokines
  - Cancer studies in vitro and animal studies

Cocoa polyphenol effects are linked

Polyphenol reduction

Influence standard cocoa process

Cocoa tree



Fermentation process

Sun-drying process

Major varieties:

1. Forastero
2. Criollo

Fresh beans upto soluble 4%PF

Forastero = 1,5 * %PF Criollo

High variations between origins

Polyphenols diffuse / sweatings

Oxidation to condensed molecules

Enzyme recations

About 30% reduction after 3 days

About 50% reduction after 6 days

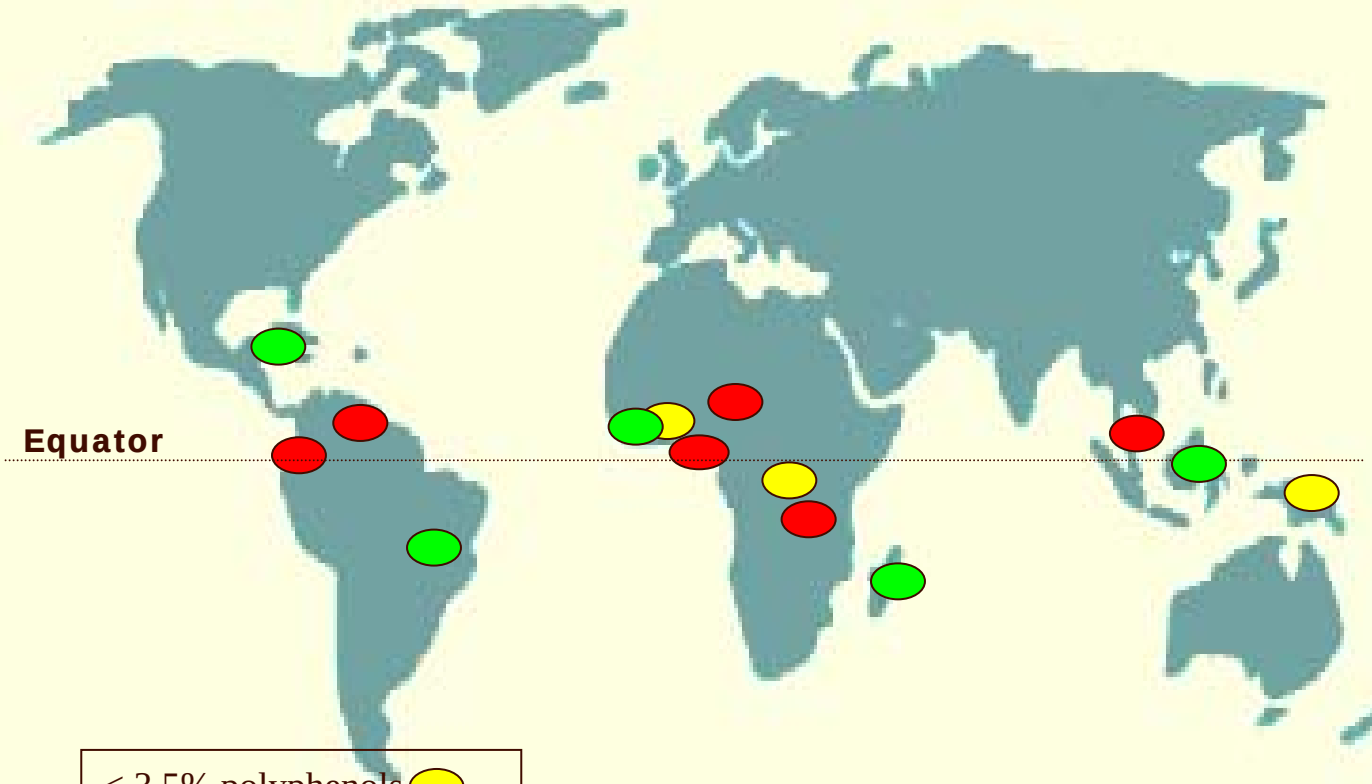
Remaining polyphenol oxidase

Upto 50% reduction in epicatechine

High impact climate

Influence of origin

Cocoa polyphenol content varies between different origins



Fermented dried beans

Origin	Polyphenol (%)
Ivory Coast	3,9 ± 0,7
Ghana	3,4 ± 0,6
Indonesia	4,6 ± 0,8
Tanzania	4,3 ± 0,9
Venezuela	4,1 ± 0,6
Ecuador	4,8 ± 0,7

Large variation between origins and within origins

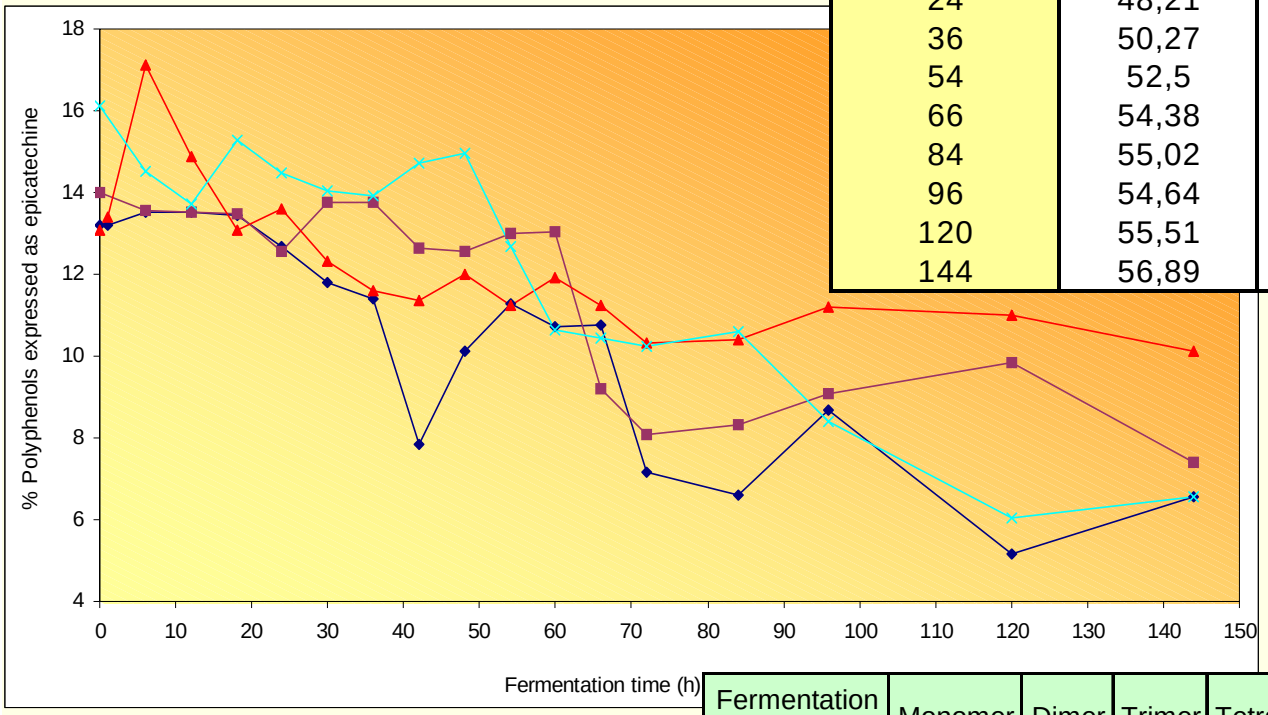
Within one origin there is a large variation, this can be more then 15% difference

Influence of fermentation on polyphenols

Upto 50% reduction in polyphenol quantity during fermentation

Fermentation time (h)	fat content (%)	Total polyphenols (mg/g)	catechin (mg/g)	epicatechin (mg/g)
0	50,93	6,9	0,43	18,48
12	50,77	6,8	0,33	16,13
24	48,21	7,07	0,49	17,74
36	50,27	6,52	0,32	14,14
54	52,5	6,26	0,29	13,51
66	54,38	6,23	0,27	10,67
84	55,02	6,34	0,37	11,61
96	54,64	5,83	0,25	8,97
120	55,51	5,57	0,27	5,64
144	56,89	4,47	0,23	5,9

-35% -45% -55%



Some studies demonstrate a 5 fold reduction in procyanidin and flavanol level during fermentation

Procyanidin (g/kg) in defatted cocoa

Fermentation time (h)	Monomer	Dimer	Trimer	Tetramer	Pentamer	Hexamer	Heptamer	Octamer	Nonamer	Total
0	21,93	10,07	10,2	7,79	5,31	3,24	1,31	0,63	0,42	60,75
24	21,09	9,76	9,12	7,06	4,74	2,91	1,36	0,61	0,36	57,25
48	20,89	9,89	9,47	7,34	4,91	2,93	1,33	0,69	0,41	58,16
96	9,55	5,78	5,06	3,36	2,14	1,16	0,46	0,25	0,14	27,91
120	8,58	4,66	4,07	2,53	1,63	0,89	0,33	0,17	0,12	22,97

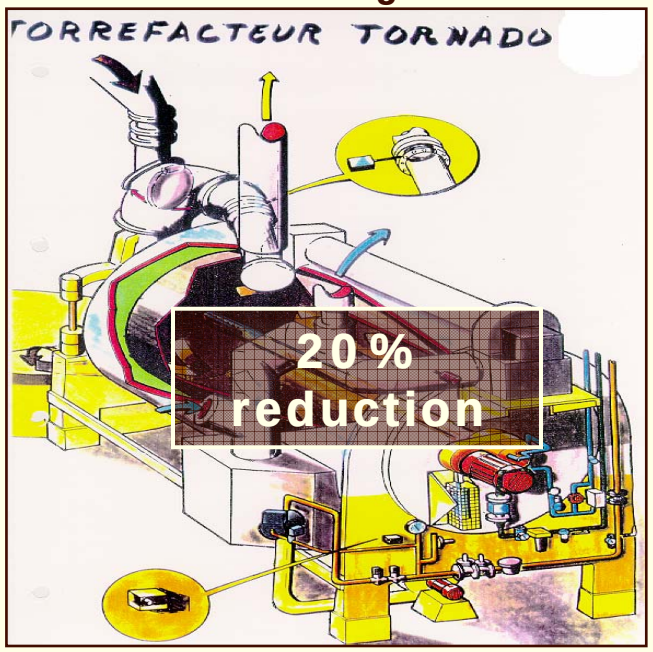
•Internal Barry Callebaut results

•Kealey et al, 1998 WO9809533

Polyphenol reduction

Influence standard cocoa process

Roasting



Roasting has a negative impact on polyphenols, reductions upto 20% are possible

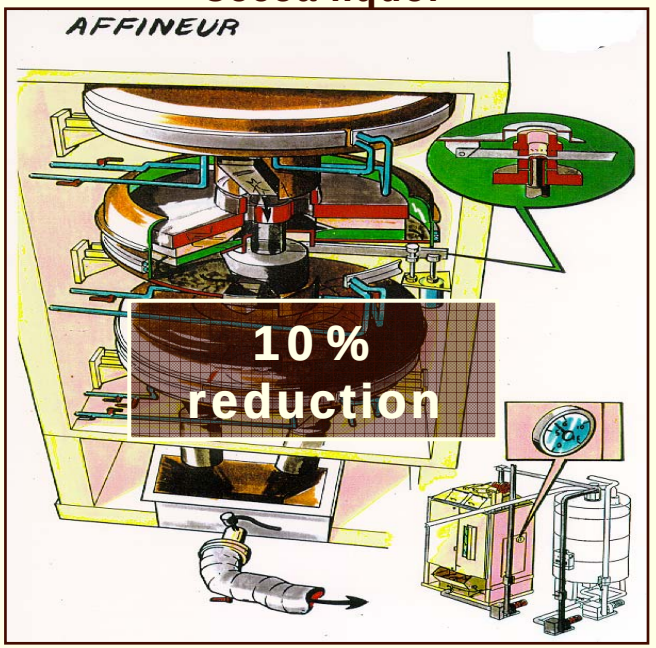
A high internal bean temperature in combination with long time, possible presence of water are important parameters

Alkalisation process

Negative effect on polyphenols, upto 30% reduction

Different parameters have an impact: temperature, alkalising agent, water %, pressure, …

Cocoa liquor



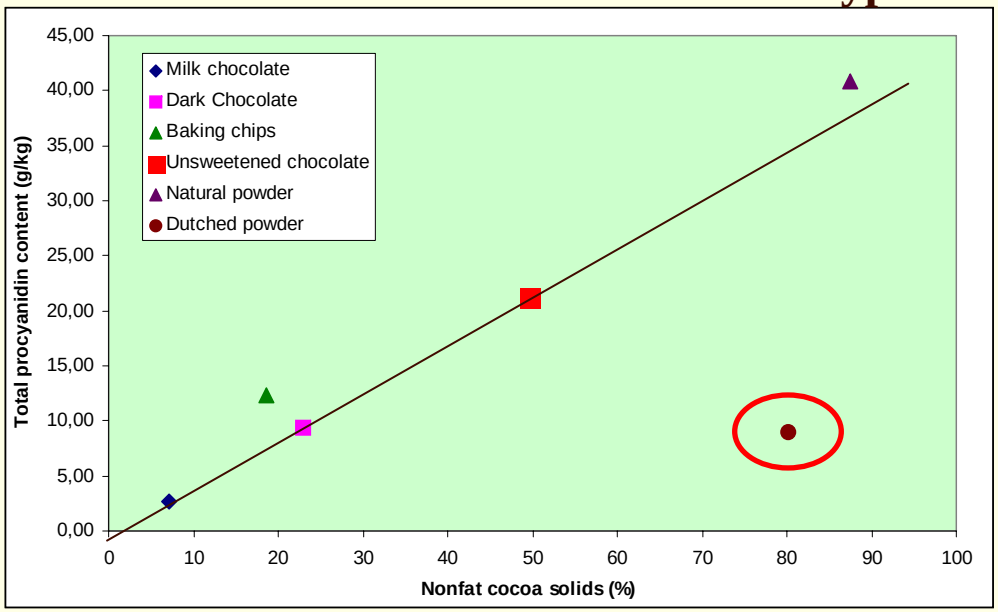
Cocoa beans are gound at higher temperature in the presence of oxygen

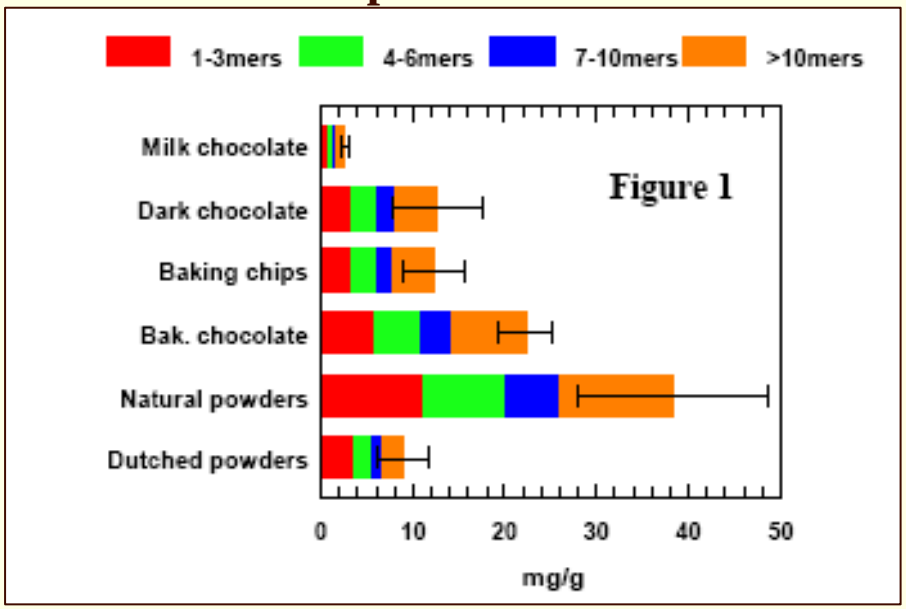
Further reduction (10%) is possible

Polyphenol reduction

Influence standard cocoa process

Correlation between nonfat cocoa solids and total procyanidin content in different types of cocoa and chocolate products





Catechin + procyanidin (g/kg) content during cocoa powder process

	Cocoa liquor	Natural cocoa powder	Dutched cocoa powder
Sample 1			,43
Sample 2			1.55
Sample 3	28.02	52.18	

Alkalisation has a huge impact on polyphenol content

Gu et al (2006), Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products, Journal of Agricultural and Food Chemistry.

Possible scenario for chocolate production

Sample	% Polyphenols	Epicatechin (mg/g)	Catechin (mg/g)	Procyanidin B1 (mg/g)	Procyanidin B2 (mg/g)	Procyanidin B3 (mg/g)
Unfermented beans	6.95	9.19	0.29	0.21	6.26	0.11
Dried fermented beans	2.69	2	0.12	0.09	1.11	<0.1
Roasting + alkalisation	1.42	0.42	0.36	0.24	0.36	0.13
Liquor production	1.23	0.34	0.27	0.15	0.35	0.11
Conching *	1.05	0.32	0.22	0.13	0.35	<0.1
Final chocolate *	1.04	0.28	0.15	0.13	0.36	<0.1

* Recalculated to cocoa liquor

Chocolate recipe:

46% sugar, 42% cocoa liquor, 11,4% cocoa butter, lecithin, aroma

The final chocolate contains less then 0,5% polyphenols

Starting from the unfermented beans, there is a polyphenol loss of about 85% !!!

Critical parameters for chocolate production

- Origin + type
- Fermentation process
- Roasting process
- Alkalisation process
- Conching

The growing conditions of the cocoa trees attribute significantly to the polyphenol content (climate, position of the cocoa pod, ...)

Reduce the sweating, the microbiological and enzymatic breakdown by removing part of the pulp and treating the beans

Reduce the high temperature and presence of water to avoid polyphenol wash-out

Limit the used temperature, water, pressure and alkali

Limit the temperature and shear during conching

Maintaining high polyphenols

Two possibilities explained:

WO2005115160A1 / US20070077318A1 / GB2414393A

Process for producing cocoa polyphenol concentrate.

Natraceutical s.a.

"….subjecting unfermented cocoa beans to a **blanching step** in water at a temperature in the range from 85-100°C for a time period in the range from 3 to 15 minutes to give unfermented cocoa beans with **reduced polyphenol oxidase activity**…."

BRPI0401999

García, Nelson Horacio Pezoa

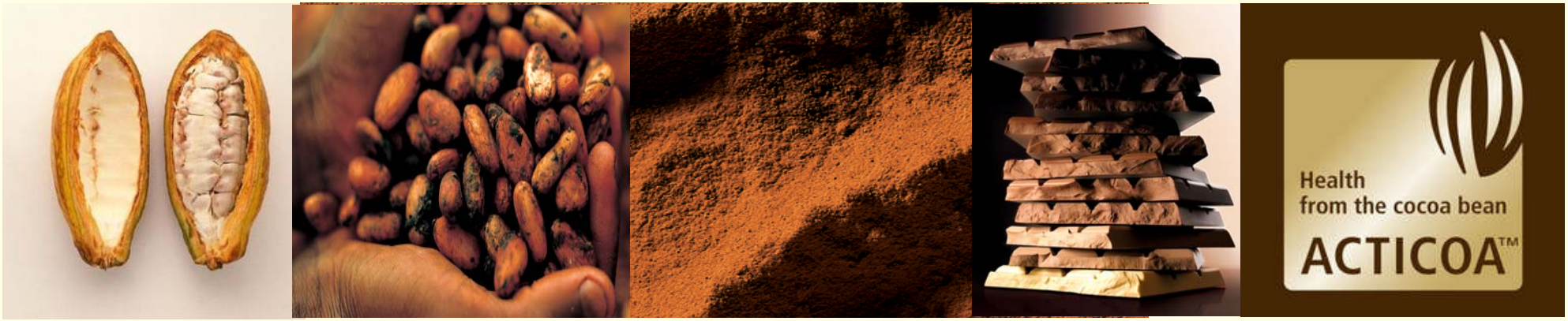
Unicamp – Brazil

…. to modify the fermentation stage of cocoa seeds to produce flavonoid-rich chocolates without prejudicing its flavor. This was done by the **inactivation of enzymes** probably responsible for flavonoid degradation, through the addition of chemical inhibitors (sodium bissulfite and cupric sulphate) during the fermentation stage … Considering the total phenolics, experiment D showed the highest retention (62,70%) from the beginning of the fermentation up to the end of the drying stage …… The chocolates B, C, D and E showed equal or better sensory acceptance as compared with conventional (A), and F and G chocolates which showed average sensory acceptance and uncertainty with respect to buying intention.

What is the impact on taste?

What is the impact on the cocoa process?

Many thanks for your attention



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