



4th International Conference and Exhibition on  
**Nutrition**  
October 26-28, 2015 Chicago, Illinois, USA

## **WORKSHOP**

**Bioactives : Case Studies**  
**Özlem TOKUSOGLU**

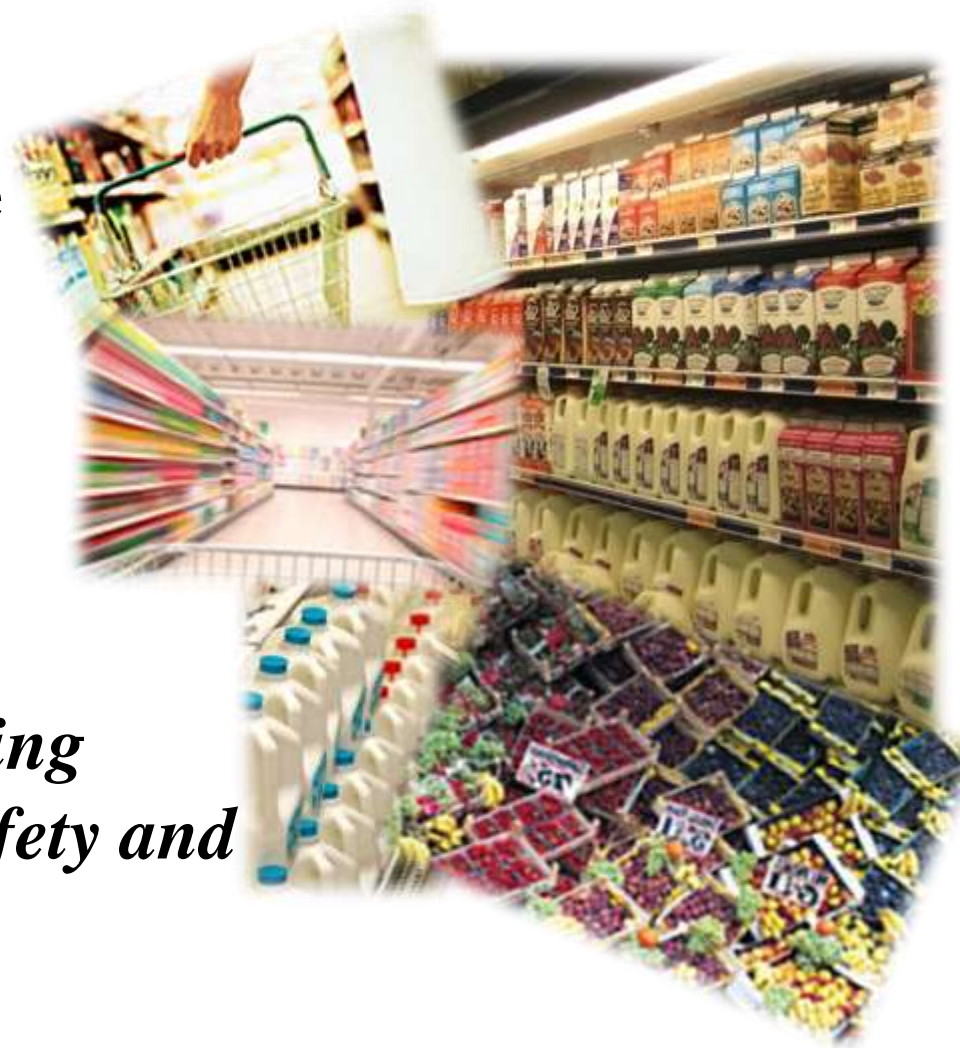
# **Presentation Flow**

- \* Pre Intro – Emerging Cold Preservation Technologies-  
Classification**
- \* HHP Effects on Bioactive Phenolics of Berry Fruits**
- \* HHP Effects on Uncommon Mycotoxigenic Bioactives  
in table olives and cheeses**
- \* HHP Effects on Allergens on Various Foods**

## *Consumer Demands*

- ❖ **With less additives**
- ❖ **With high nutritional value**
- ❖ **High quality**
- ❖ **Less thermal damage**
- ❖ **Good sensory properties**
- ❖ **Safe products**

*Thereby, food manufacturing designed for better food safety and quality.*



# Strategies for Food Processors



- Premium food products
- Long lasting Foods
- Convenience foods
- Minimally processed foods
- Ready-to-cook meals
- Ready-to-eat foods
- Low-fat foods
- Low-carbohydrate foods
- Specialities in foods

(For Health Treatments

For Kids

For Military

For Pregnants

For Sportmans)



**NONTHERMAL**

- High Hydrostatic Pressure
- Pulsed electric fields
- Ultrasound
- Ultraviolet
- Irradiation
- Cold Plasma
- DensePhase CarbonDioxide
- Ozone
- Chemicals

**THERMAL**

- Microwave
- Radiofrequency
- Ohmic Heating
- Induction Heating

# Fruit and Nut Bioactives

**Phytochemical  
Bioactives**

✓ *Polyphenols*

✓ *Carotenoids*

**Bioactive  
Lipids**

**Mycotoxigenic  
Secondary  
Metabolite Bioactives**

*Health-Promoting,  
and /or Nutraceutical  
and Medicinal Proper*

*Toxic Substances*

# Phytochemicals

**As the name suggests, phytochemicals working together with chemical nutrients found in fruits, cereals, and nuts may help slow the aging process and reduce the risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, and urinary tract infections**

**REF: (Meskin et al. 2003; Omaye et al. 2000).**

# PHENOLIC COMPOUNDS IN FOODS



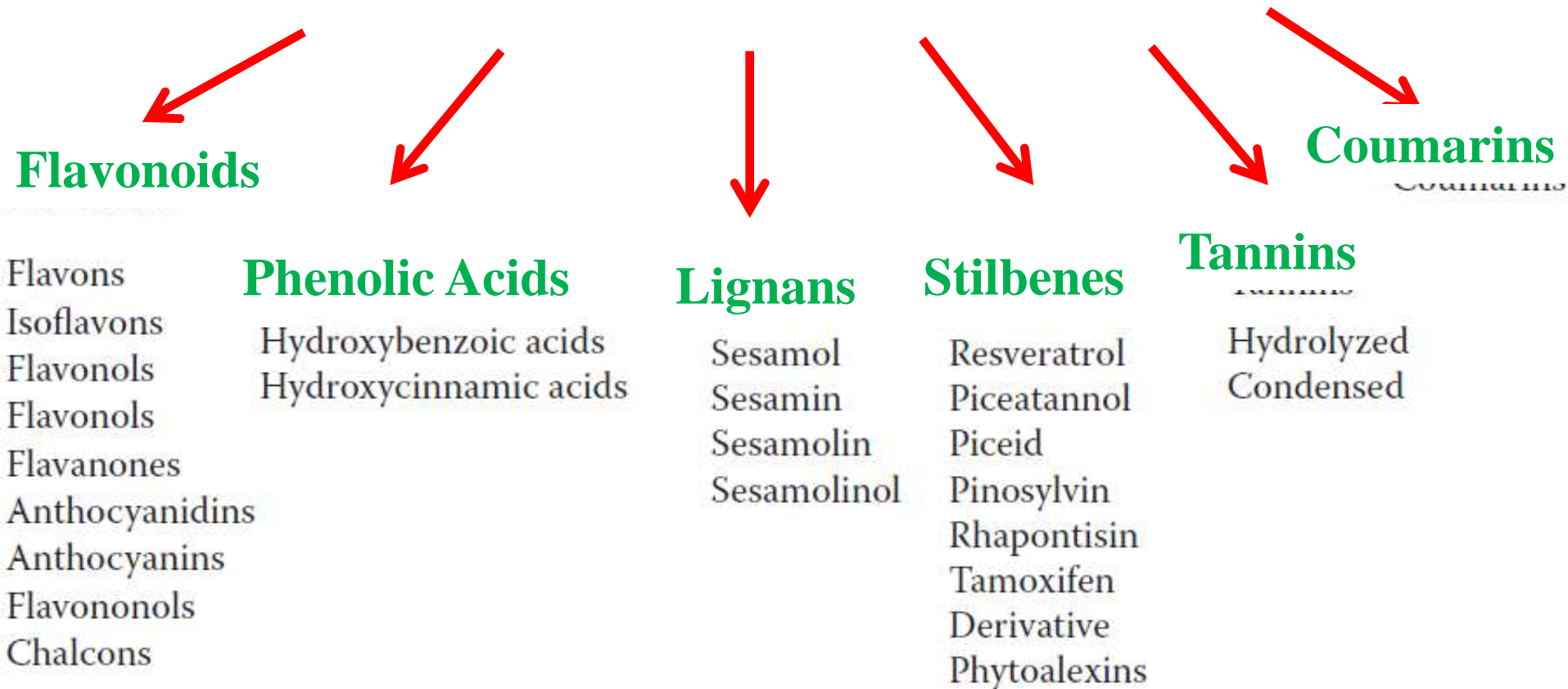
Phenolic compounds are important components of many fruits, vegetables and beverages due to their contributing to flavor, color and sensory properties (as bitterness and astringency).

Especially recent interest in functional foods and medicinal utilization of phenolics have also increased interest in their chromatographic separation.

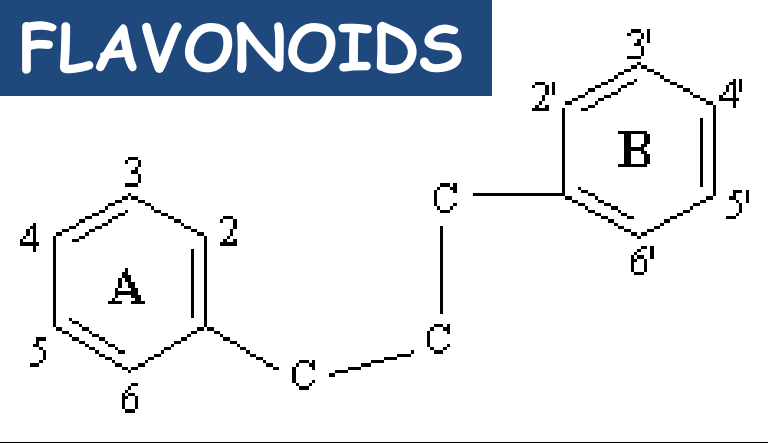
- Aromatic ring and attached many of hydroxyl (-OH) groups abundantly are formed the phenolic compounds.



# Phenolic Compounds in Foods



# FLAVONOIDS



( $C_6C_3C_6$  structure)

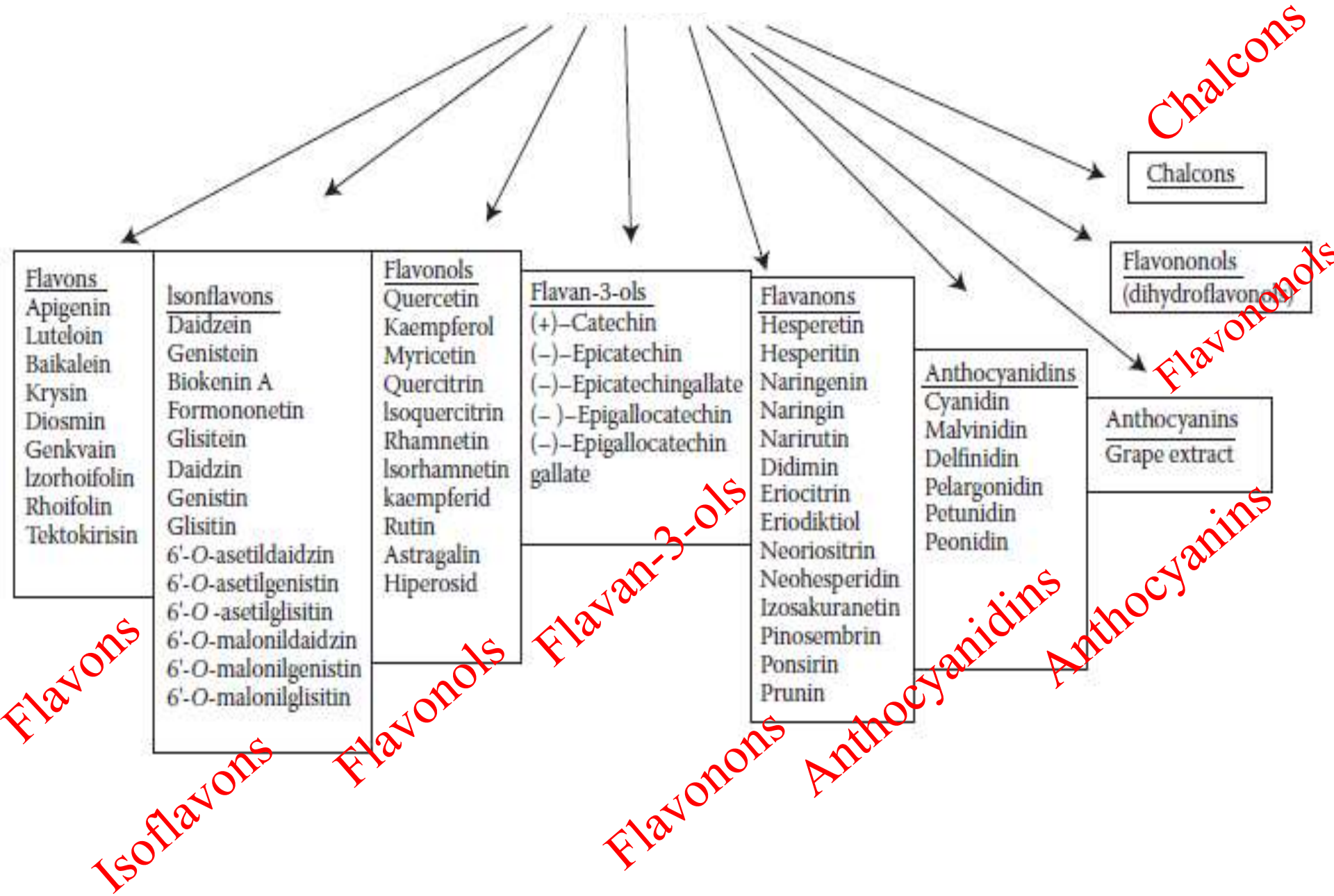
General Structure of flavonoids contain 2-phenyl-benzopyrane ring ( $C_6C_3C_6$ ) and secondary metabolites of plants.

Majority of the polyphenols are flavonoids.

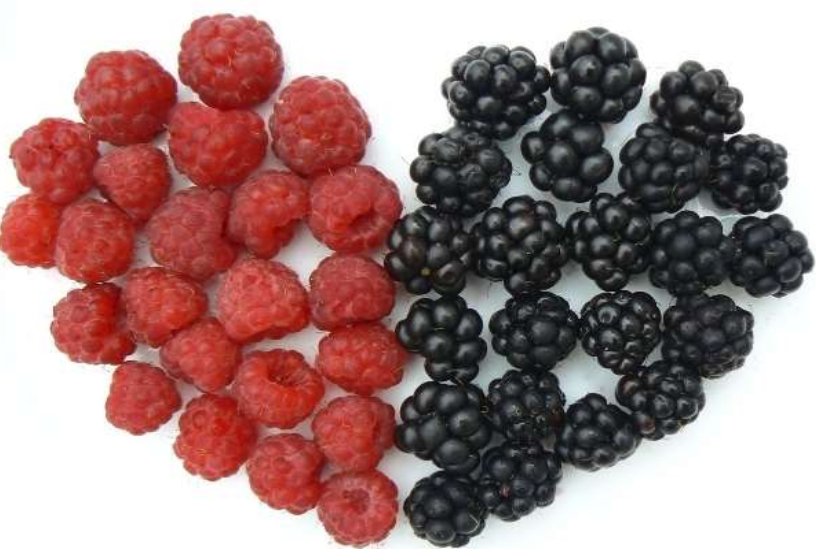
General Properties of Flavonoids; They are based on the conformation of a heterocyclic diphenyl propane ring and characterized as 9 subclass: flavons, isoflavons, flavonols, flavanols, flavanons, antosiyanidins, antosiyanins, flavononols and chalcones.



# Flavonoids



**Berry Phenolics & HHP Effects**



# Berry Phenolics



**Berry fruits, commonly called aggregate fruits, have clusters of one-seeded drupelets, each cluster of drupelets developing from a single flower.**

**Berry is a term used for a fruit having succulent pericarp. Berry fruits are a simple type of fruit that are fleshy or succulent at maturity.**

**Blackberry** (*Rubus fruticosus* sp.)

(*drupacetum achenecetum type aggregate berries*)

**Bluberry** “highbush” blueberry (*V. Corymborsum*, *V. ashei*),  
“lowbush” blueberry (*V. augustifolium*),

**Raspberry (Red, Black)** (*Rubus idaeus* L.)

(*drupacetum achenecetum type aggregate berries*)

**Strawberry** (*Fragaria X ananassa* Duch)

(*achenecetum type aggregate berries*)

**Cranberry** (*Vaccinium macrocarpon*)

**Huckleberry** (*Vaccinium parvifolium*)

**Bayberry** (*Myrica rubra* Sieb. et Zucc.)

**Chokeberry** (*Aronia melanocarpa*),

**Currant** (*Ribes rubrum*, *R. Petraeum* and *R. sativum*),

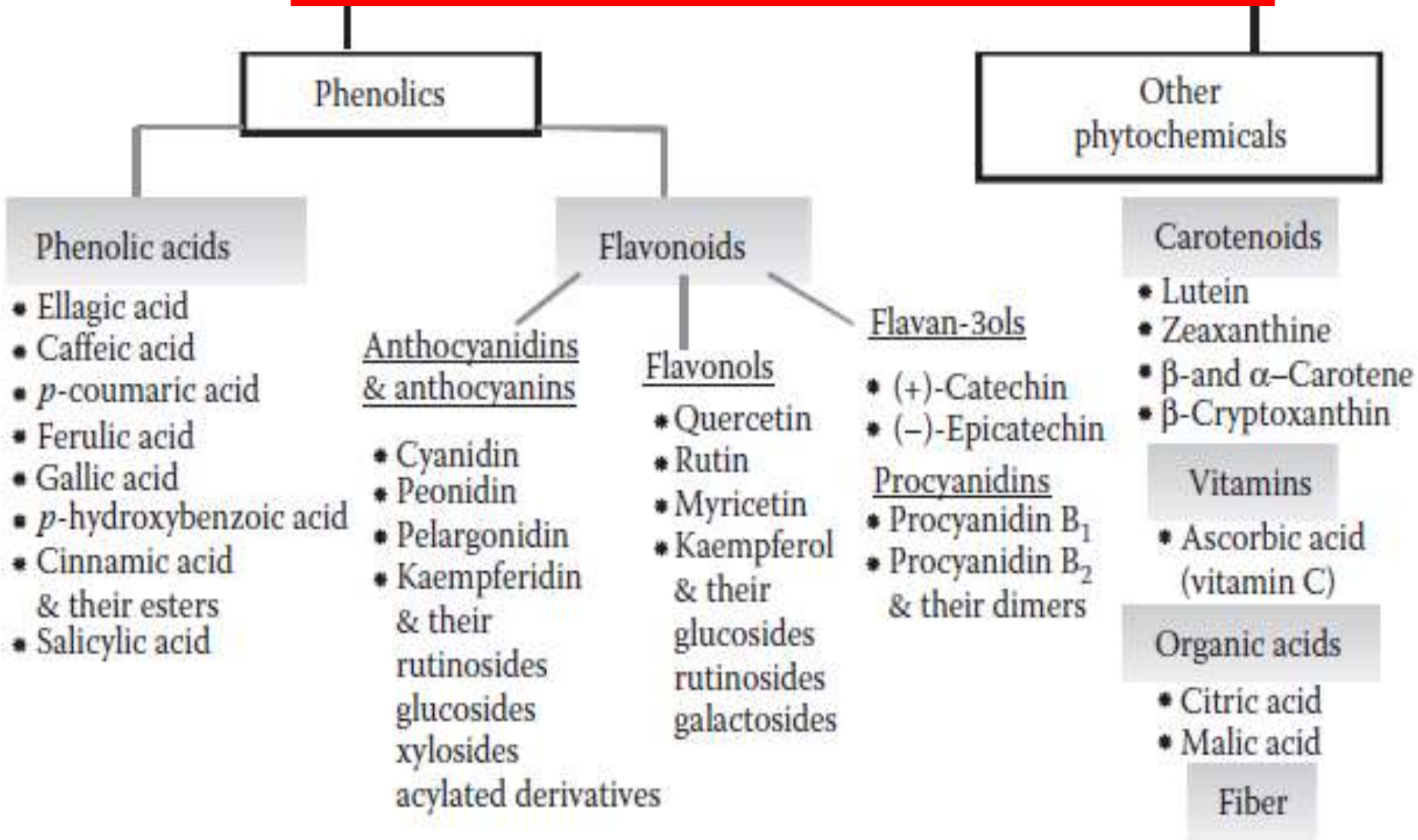
**Elderberry** (*Sambucus nigra* or *Sambucus simpsonii*),

**Gooseberry** (*Ribes uva-crispa* or *Ribes grossularia*)

*Simple  
aggregate  
berries*



# Major Bioactives in Berry Fruits



REF: Tokuşoğlu & Stoner . 2011. In *Fruit and Cereal Bioactives: Sources, Chemistry & Applications*. 2011. ISBN: 9781439806654, 459 p. *CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA*



## High pressure processing

**High pressure processing (HHP) is nonthermal technique of foods has been revealed as a useful tool to extend their shelf-life and quality, it is a novel food preservation technique for microbial and enzyme inactivation and as well as to preserve their nutritional and functional characteristics in comparison with those of traditional thermal processing results.**



**Consumer perception of food quality depends not only on microbial quality, but also on other food factors such as biochemical and enzymatic reactions and structural changes.**

**In this context, HPP can have an effect on food yield and on sensory qualities such as food color and texture.**

**High pressures can also be used to enhance extraction of compounds from foods.**



## **SOURCES**

*(Cheftel, 1995; Hogan et al.,2005)*

## High Pressure Processing (HPP)

- ▶ HPP conditions in the range of 300 – 700 MPa at moderate initial temperatures (around ambient)
- ▶ Sufficient to inactivate vegetative pathogens for pasteurization processes, some enzymes, or inactivate spoilage organisms to extend shelf-life.

Conditions of Inactivation of spoilage organisms to extend shelf-life (and provide extra assurance against pathogens)

To inactivate bacterial spores such as *Clostridium botulinum* for the production of ambient shelf-stable low-acid foods requires high pressure-high temperature combinations.

Such processes typically involve high pressures in the range of 600 – 800 MPa and higher initial temperatures around 80 – 90 °C.

# **Advantages of High Pressure Processing (HPP)**

During pressurization, rapid adiabatic heating generates temperatures above 121 °C. This process to achieve commercial sterility in low-acid foods is called “Pressure-assisted Thermal Sterilization” and has several technical advantages over conventional thermal sterilization methods

- ✓ shorter processing times,
- ✓ improved food quality,
- ✓ increased energy efficiency
- ✓ pressure is transmitted uniformly throughout the package and product, the food retains its original shape.

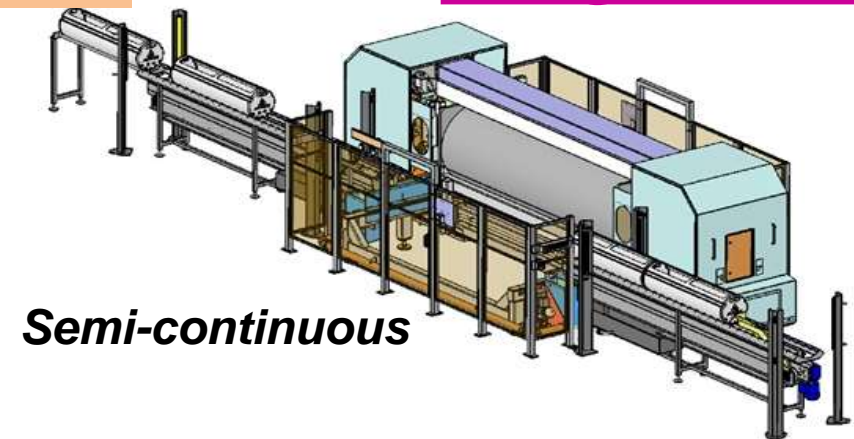
**Systems cost in the range of \$0.5 – 2.5 million, depends on**

- ▶ the size of the vessel,
- ▶ extent of automation,
- ▶ other design features

HPP process works particularly well for unstructured foods containing water, whereas foods with internal air pockets (strawberries, marshmallows, some bakery items) tend to collapse, and dry solids tend not to have enough moisture to allow efficient microbial destruction. When the product is removed from the high pressure vessel, the package is covered with water.



**C** *Large-scale HPPs*



▶ There are a number of high pressure equipment manufacturers worldwide making HPP equipment for food preservation

**Evidence is emerging from clinical, animal researches that phytochemicals and bioactive compounds containing especially polyphenols (flavonoids, anthocyanins etc.), carotenoids, functional lipids might offer greater protection against chronic diseases when acting in combination rather than individually.**

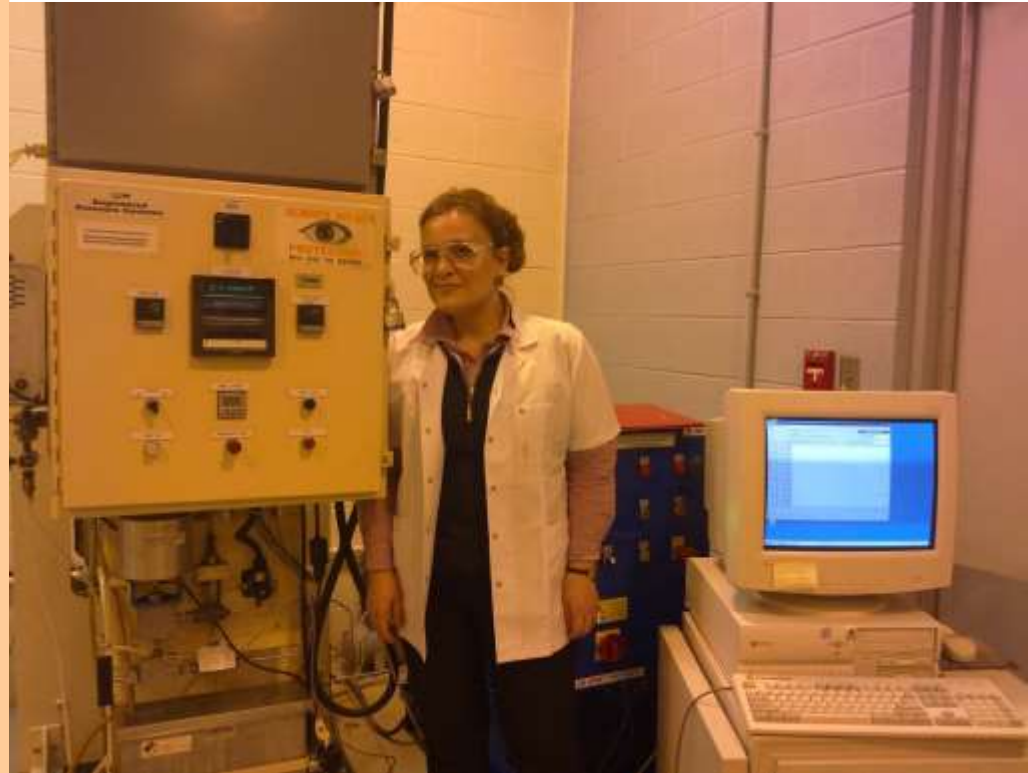


**Recent studies showed that HHP preserve phytochemicals and minor food bioactives without the quality, quantity, damage caused by heat treatments. It would appear from a nutritional prospective, HHP offer high stability of phytochemicals and the great potential of retain bioactives with health properties in foods.**

**Recent studies have shown that high pressure extraction (HPE) can shorten processing times, and provide higher extraction yields while having less negative effect on the structure and antioxidant activity of bioactive constituents.**

**The use of HPE enhances mass transfer rates, increases cell permeability, and increase diffusion of secondary metabolites.**

**HPP can also be increased the extraction capacity of phenolic constituents, and higher levels of bioactive compounds are preserved in HPP-treated samples.**



*HPP Unit in Washington State Univ. Dept of Food Eng.  
April, 2010 - Pullman - Washington*

*(Sources:*

*Tokuşoğlu & Doona, 2010; Tokuşoğlu, Alpas & Bozoğlu, 2010; Ahmed & Ramaswamy, 2006; Zhang et al. 2004, 2005ab; Dornenburg & Knorr 1993; Richard, 1992)*

# HPP Effects on Antioxidant Phenolics and Antioxidant Activity

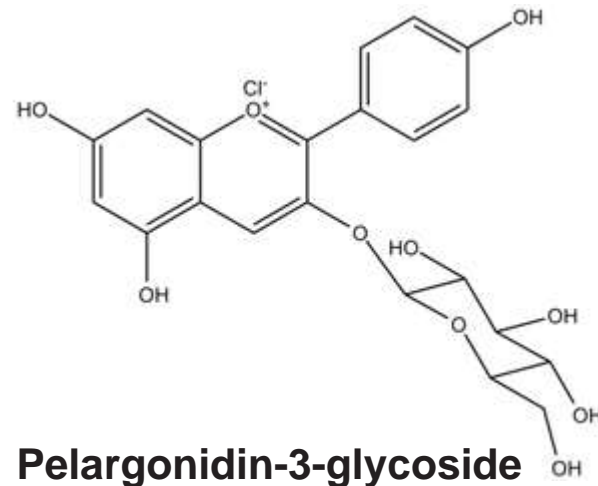
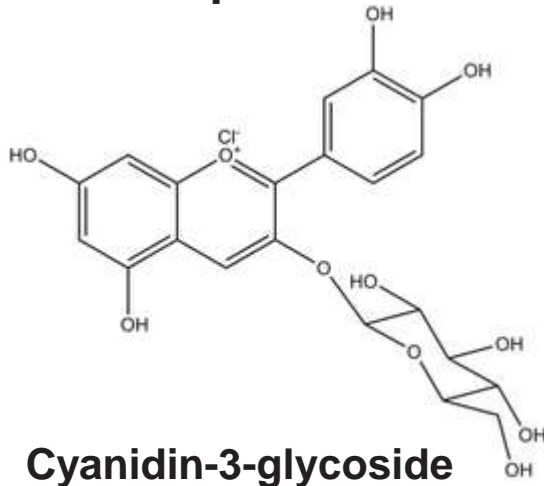


The effect of HPP treatments and conventional thermal processing on antioxidant activity, levels of bioactive antioxidant compounds (polyphenols, ascorbic acid and anthocyanins), and the color of strawberry and blackberry purées



Blackberry Purée Strawberry Purée

Key antioxidants (cyanidin-3-glycoside, pelargonidin-3-glucoside, and ascorbic acid) in strawberry and blackberry purées and the antioxidant activity of these purées were quantified after various HPP treatments





**Table 1.** The Antioxidant Indices of HPP-treated and Thermally Processed strawberry and blackberry purées

Treatment	Anti-radical power (g/L) <sup>-1</sup>		Total phenols, mg GAE/100g DW <sup>e</sup>		Anthocyanin, mg/100g DW		Ascorbic acid, mg/100g DW	
	Strawberry	Blackberry	Strawberry	Blackberry	Strawberry <sup>f</sup>	Blackberry <sup>g</sup>	Strawberry	Blackberry
<b>Unproces.</b>	1.55± 0.07 <sup>a</sup>	2.86± 0.23 <sup>a</sup>	855.02± 6.52 <sup>a</sup>	1694.19± 3.0 <sup>a</sup>	202.27± 0.50 <sup>a</sup>	1004.90± 8.60 <sup>a</sup>	633.10± 9.31 <sup>a</sup>	nd
<b>Thermal</b>	1.16± 0.01 <sup>b</sup>	2.78± 0.26 <sup>a</sup>	817.01± 5.26 <sup>b</sup>	1633.62± 8.4 <sup>a</sup>	145.82± 6.40 <sup>b</sup>	975.28± 7.90 <sup>b</sup>	496.11± 0.04 <sup>b</sup>	nd
<b>HPP (400 MPa)</b>	1.25± 0.05 <sup>b</sup>	3.87± 1.11 <sup>a</sup>	859.03± 6.56 <sup>a</sup>	1546.26± 8.0 <sup>a</sup>	173.34±6.5 1 <sup>ab</sup>	1039.21± 4.51 <sup>a</sup>	574.30± 3.93 <sup>c</sup>	nd
<b>HPP (500 MPa)</b>	1.30± 0.02 <sup>ab</sup>	3.70± 0.57 <sup>a</sup>	926.00± 5.93 <sup>a</sup>	1724.65± 0.7 <sup>b</sup>	202.53± 5.40 <sup>a</sup>	1014.21± 0.10 <sup>a</sup>	577.10± 6.52 <sup>c</sup>	nd
<b>HPP (600 MPa)</b>	1.33± 0.02 <sup>a</sup>	4.80± 1.79 <sup>b</sup>	939.01± 0.99 <sup>c</sup>	1778.44± 6.0 <sup>b</sup>	204.30± 1.60 <sup>a</sup>	1014.47± 1.00 <sup>a</sup>	599.11± 0.60 <sup>c</sup>	nd

Values are mean ± standar deviation, n=3, mean values in a column with diffent letters are significantly different at p<0.05; nd= not detected

<sup>e</sup>Dry weight

<sup>f</sup>Expressed as mg/100g DW pelargonidin-3-glucoside.

<sup>g</sup>Expressed as mg/100g DW cyanidin-3-glucoside.



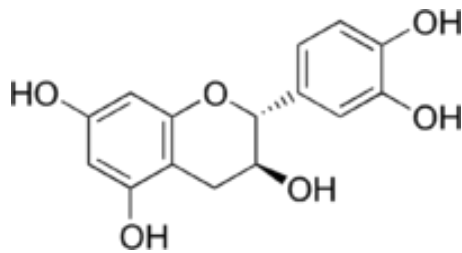
**400, 500, 600 MPa/15 min/10–30 °C) and thermal treatments (70 °C/2 min). Table 1 shows the antioxidant indices of HPP-treated and thermally processed strawberry and blackberry purées**

- ✓ **The three different pressure treatments did not cause any significant changes in ascorbic acid levels.**
- ✓ **Following thermal processing ( $P_{70} \geq 2$  min), the ascorbic acid content degraded by 21% compared to the unprocessed purée.**
- ✓ **Similarly, no significant changes in anthocyanin compounds were observed in HPP-treated and unprocessed purées, while conventional thermal treatments significantly reduced the anthocyanin levels**
- ✓ **Antioxidant activity of HPP-treated strawberry and blackberry purées were significantly higher than in thermally processed purées**

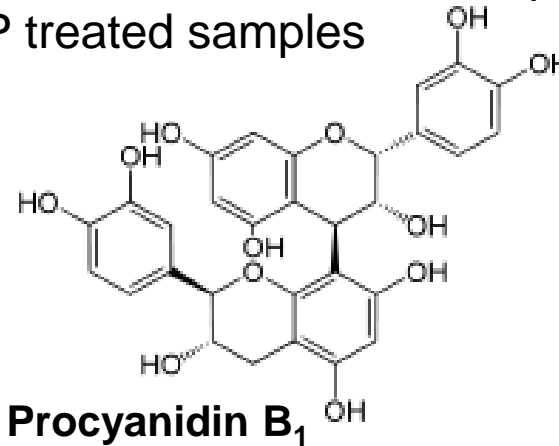


# HHP Effects on total phenolics, major polyphenols (Procyanidin B<sub>1</sub>, catechin), antioxidant activity, microbial quality in grape pomaces

- ✓ High Pressure (500 MPa, 30 min) and also ultrasound effects on procyanidin B<sub>1</sub> -catechin alteration and microbiological quality detection of 10 varieties of grape pomaces (*Alicanthe Buche*, *Merlot*, *Öküzgözü*, *Kalecik Karası*, *Boğazkere*, *Ugniblanc*, *Cabernet Sauvignon*, *Emir*, *Syrah*, *Narince*) were carried out.
- ✓ In HHP treated pomace samples, antioxidant activity, total phenolic levels increased (due to extraction capability rised). Catechin concentration increased in HHP treated and ultrasound treated samples. Microbial stability was highly preserved in HHP treated samples



Catechin



Procyanidin B<sub>1</sub>



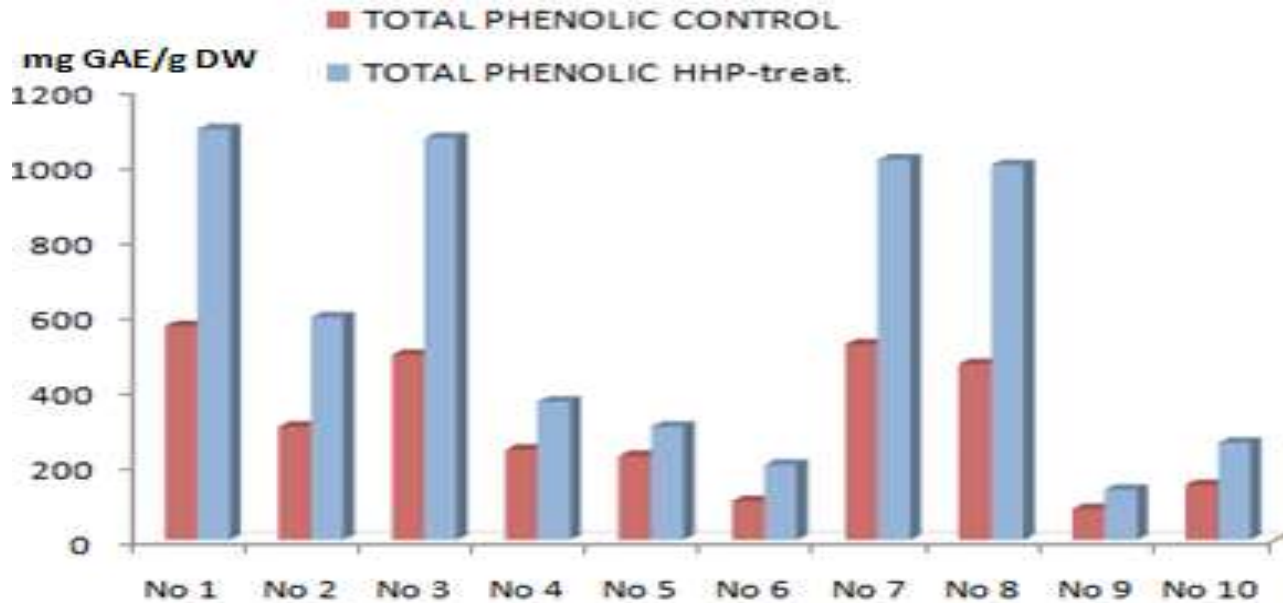
It is stated that (+)-catechin (Cat), epicatechin (Epicat), procyanidin dimmers ( $B_1$ - $B_4$ ) and trimers in grape skin and seed.

**SKIN:** It had been determined that  **$B_1$  dimer is dominant (64%)** in grape skin. Besides, it was detected that **(+)-catechin (Cat) level was 4 fold more** than epicatechin (Epicat) amount in grape skin





# TOTAL PHENOLIC



- No 1 pom. Merlo
- No 2 pom. Öküzg.
- No 3 pom. kal. kar
- No 4 pom. Boğaz
- No 5 pom. Uğnib
- No 6 pom. Emir
- No 7 pom. Ciber
- No 8 pom. Alice
- No 9 pom. Syrah
- No 10 Pom. Narince

# ANTIOXIDANT ACTIVITY



- No 1 pom. Merlo
- No 2 pom. Öküzg.
- No 3 pom. kal. kar
- No 4 pom. Boğaz
- No 5 pom. Uğnib
- No 6 pom. Emir
- No 7 pom. Ciber
- No 8 pom. Alice
- No 9 pom. Syrah
- No 10 Pom. Narince



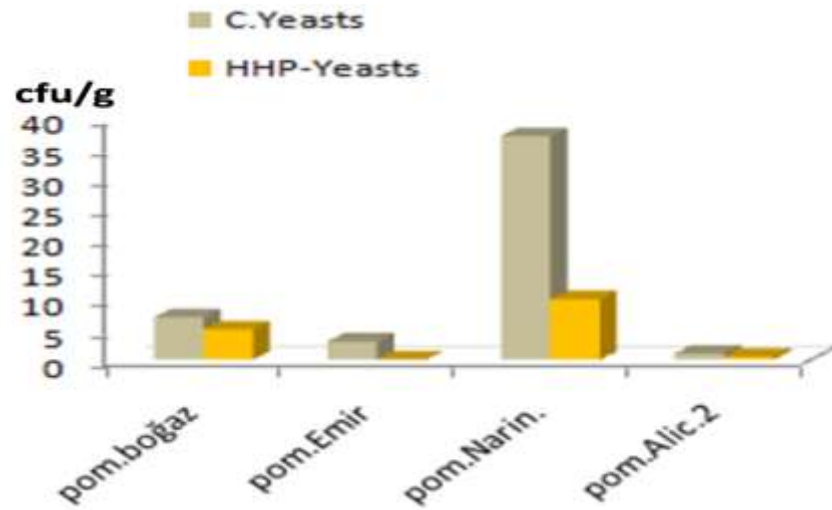
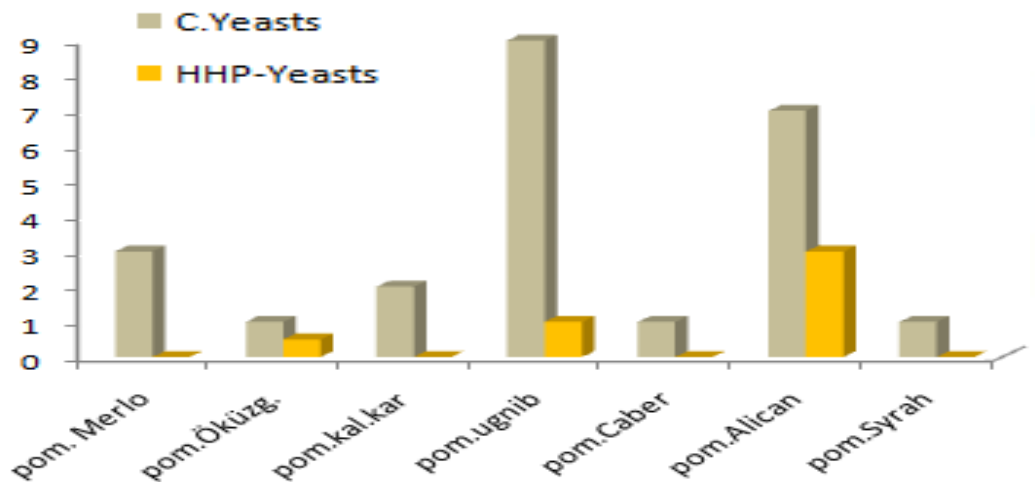
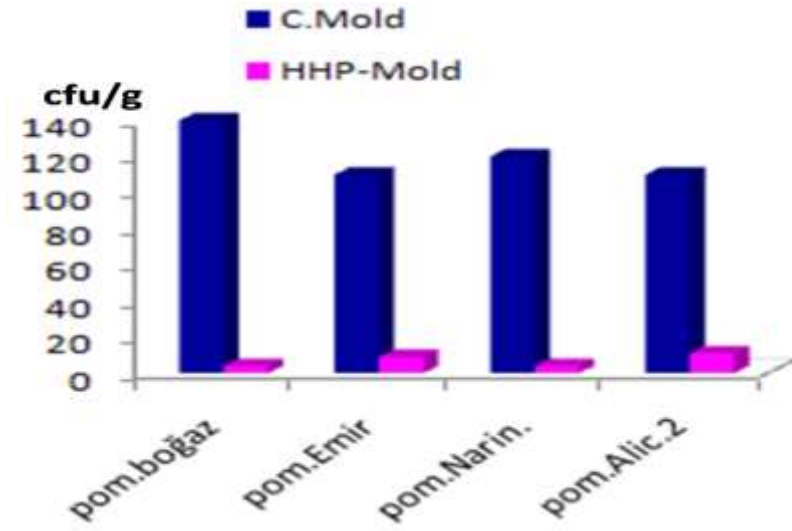
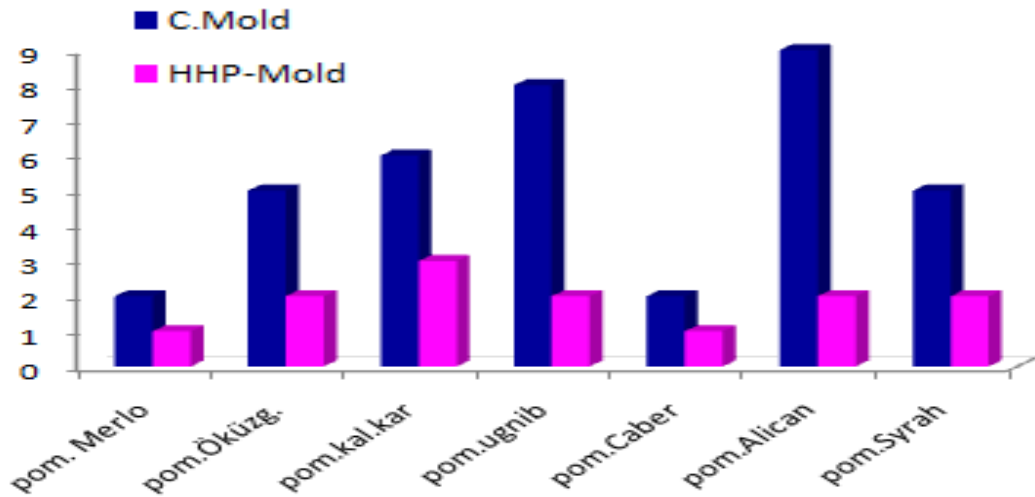
With HHP application of pomaces, total mold and yeast load was reduced more than 95% at 25 ° and total plate count (TPC) was reduced more than 95%.

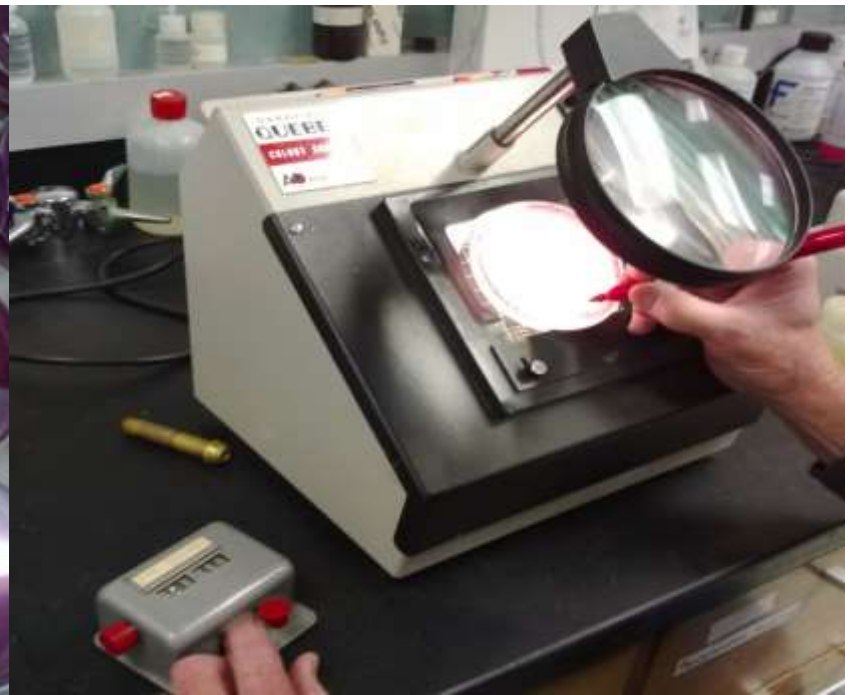
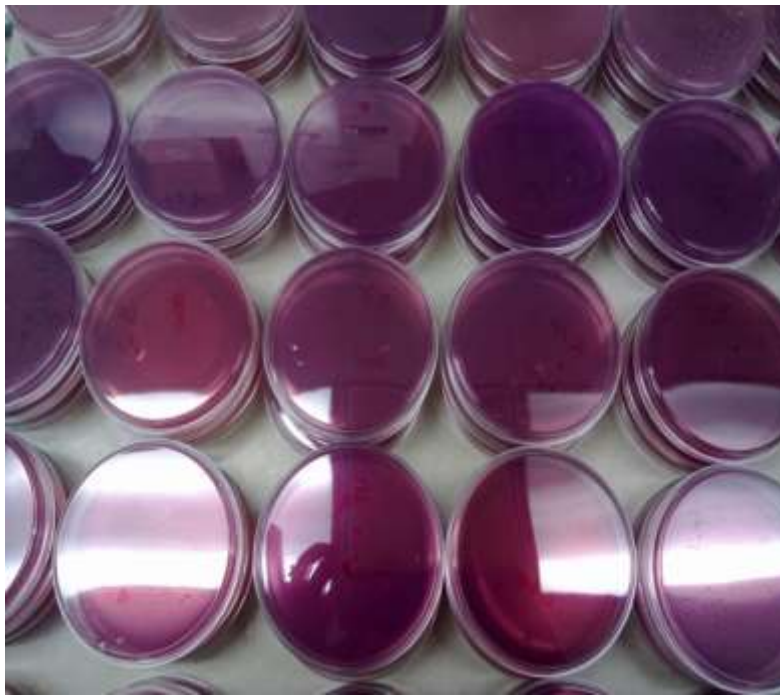
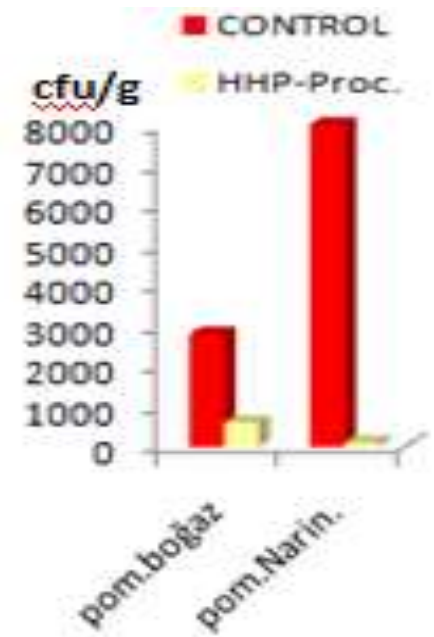
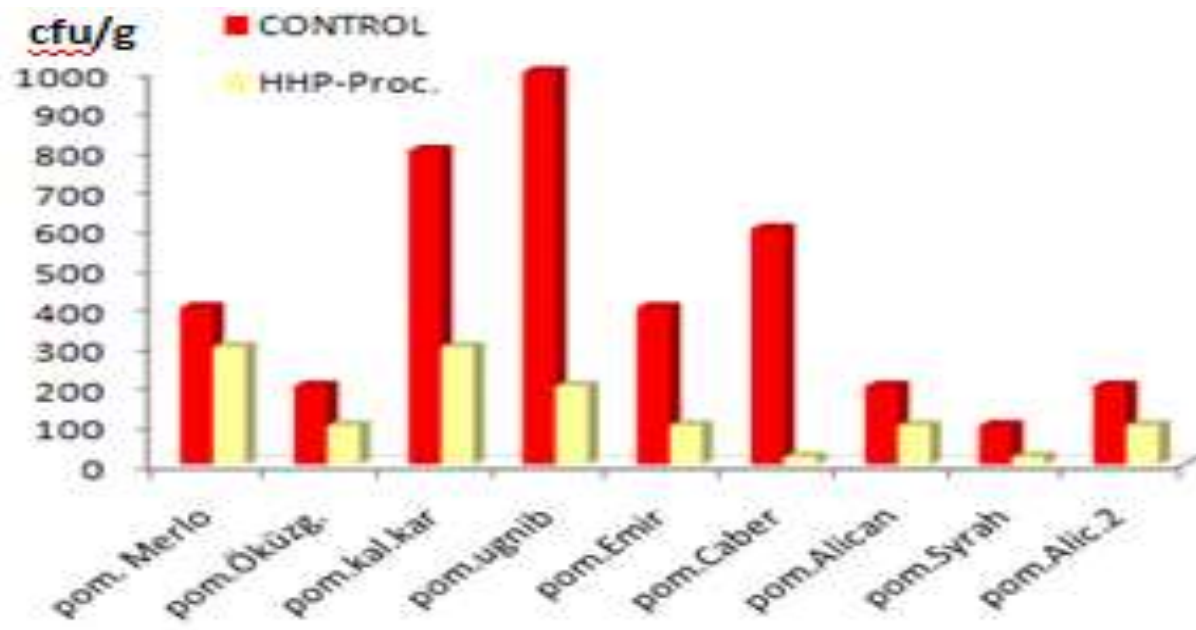
**Antioxidant activity (AA) increased 1.22-1.98 fold after HHP processing.**

**Total Phenolics (TPs) increased 1.35-2.16 fold after HHP processing. The correlation between the TP control and TP-HHP processed was found very high for all samples ( $R^2=0.9635$ ) ( $y= 2.1386x - 78.103$ )**

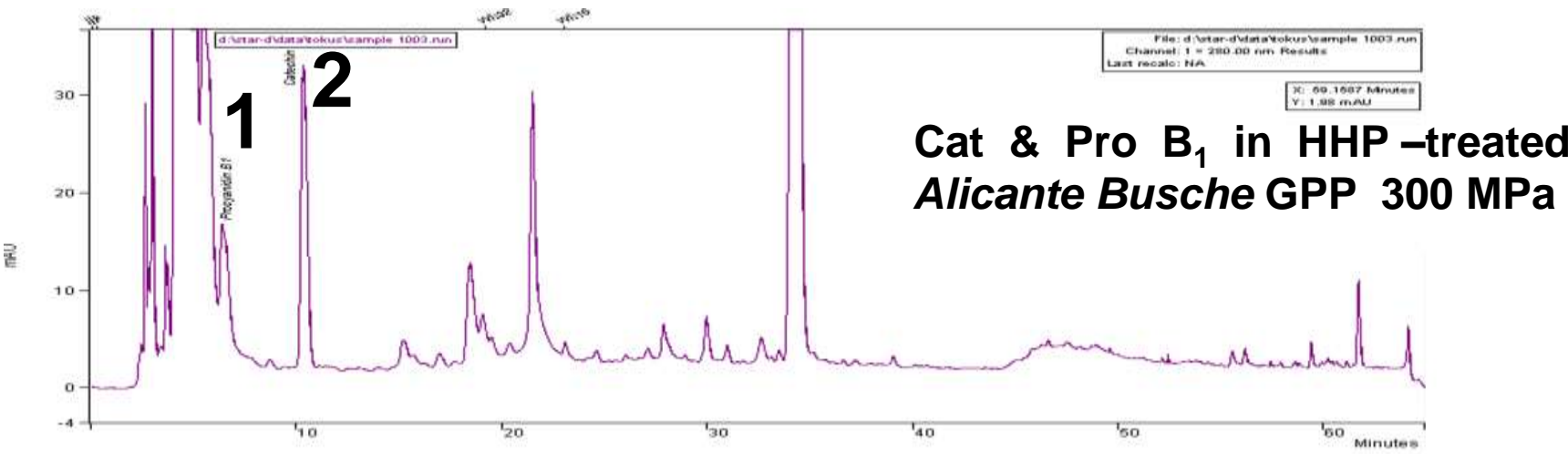
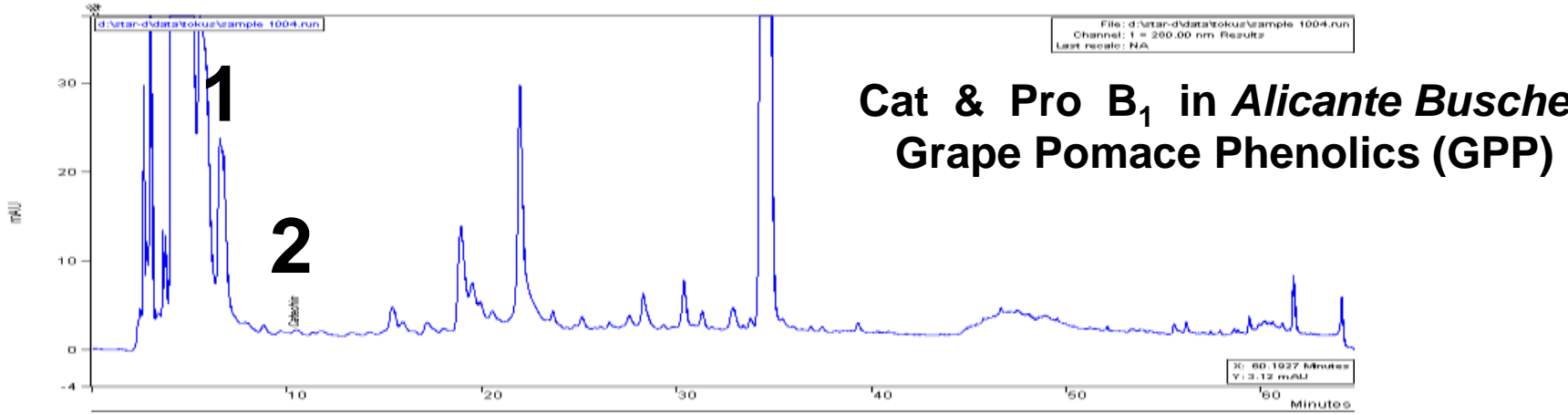
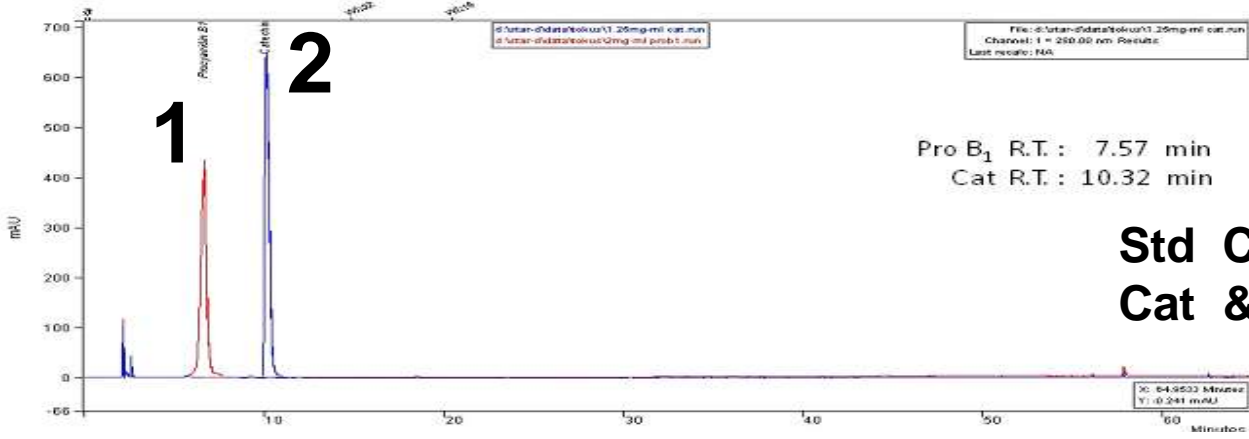


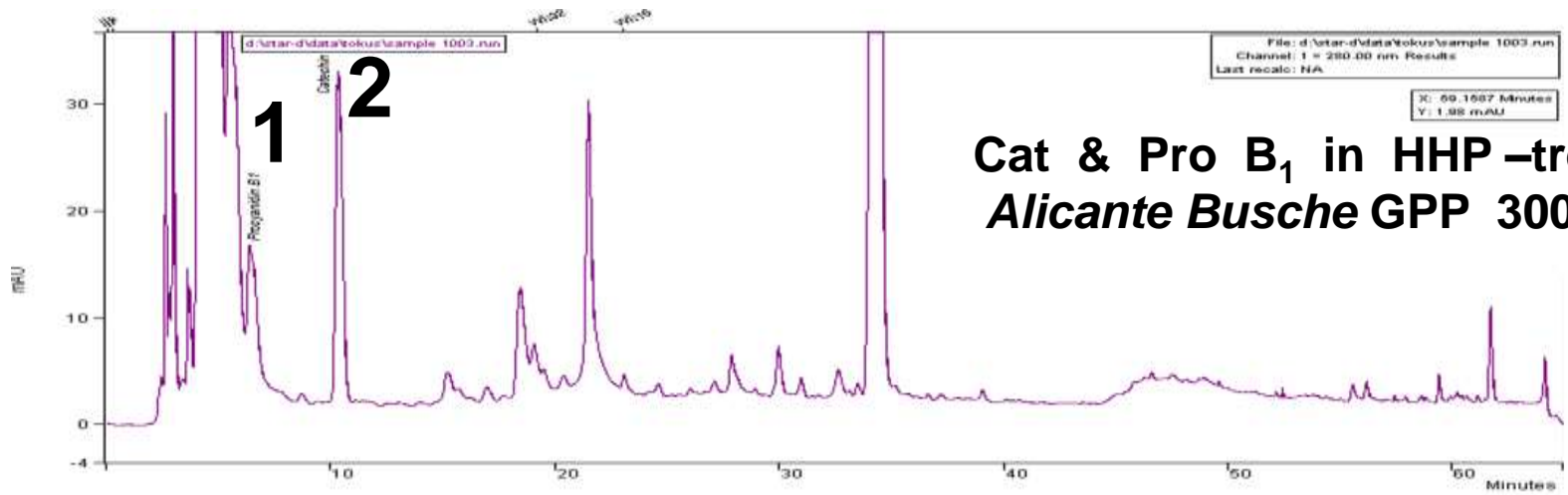
# MICROBIAL QUALITY FOR HHP PROCESSED GRAPE POMACES



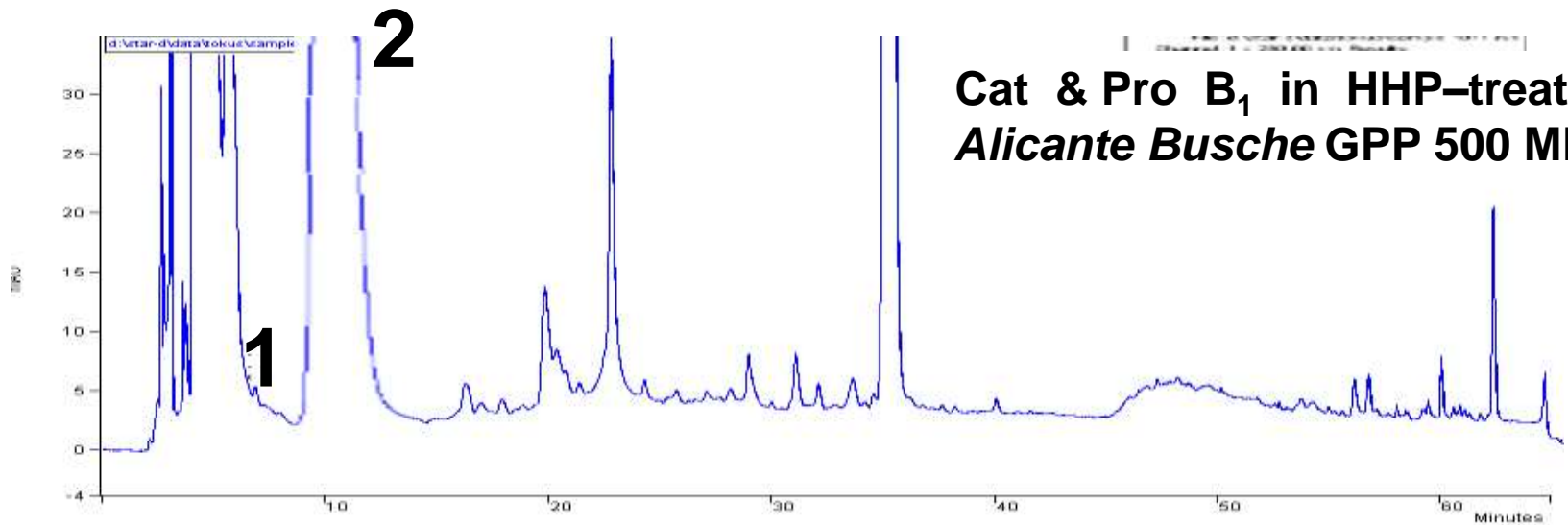








**Cat & Pro B<sub>1</sub> in HHP –treated  
*Alicante Busche GPP 300 MPa***

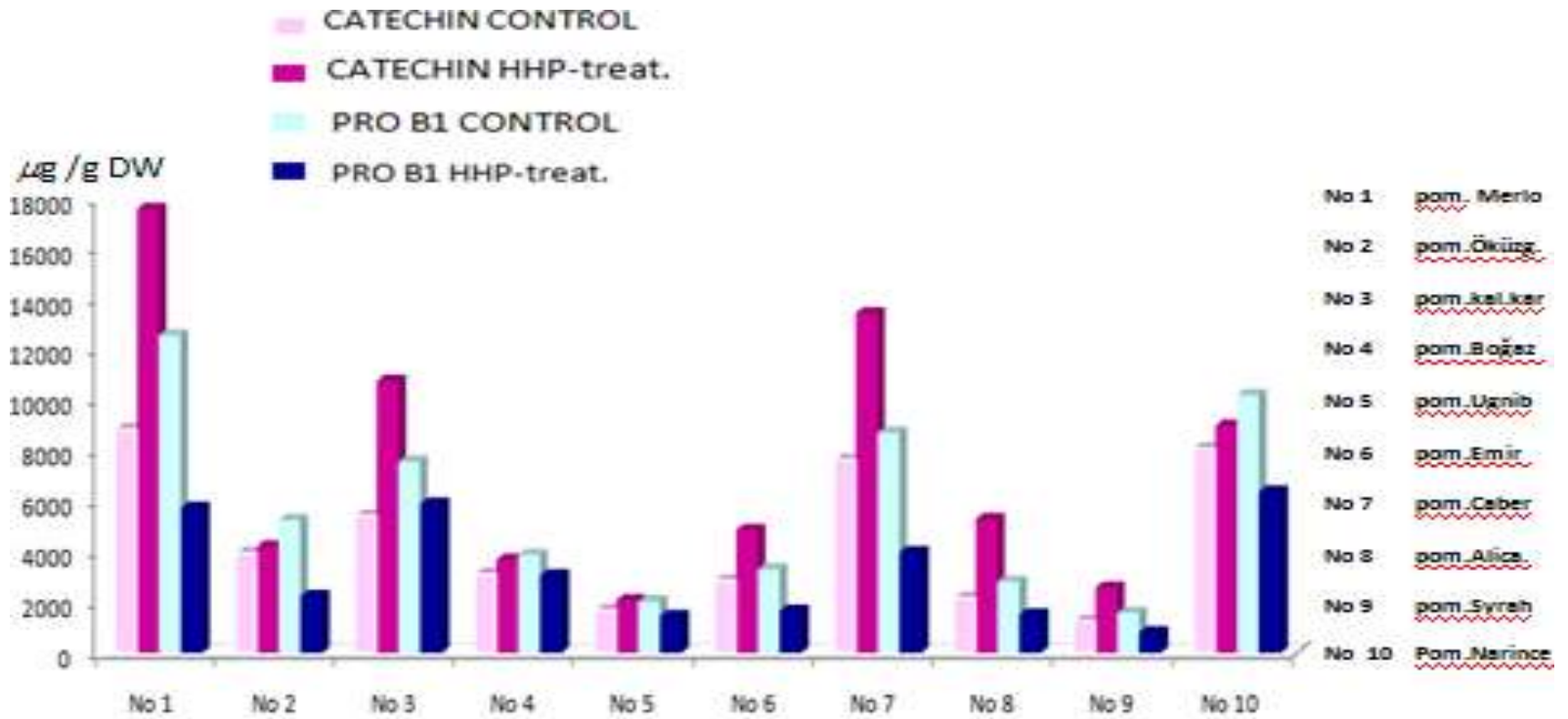


**Cat & Pro B<sub>1</sub> in HHP–treated  
*Alicante Busche GPP 500 MPa***

**Procyanidin B<sub>1</sub> (Pro B<sub>1</sub>) phenolic decreased 1.27- 2.34 fold after HHP processing (+)-Catechin (CAT) phenolic increased 1.11 - 3.42 fold after HHP processing.**



# CATECHIN / PROCYANIDIN B<sub>1</sub>

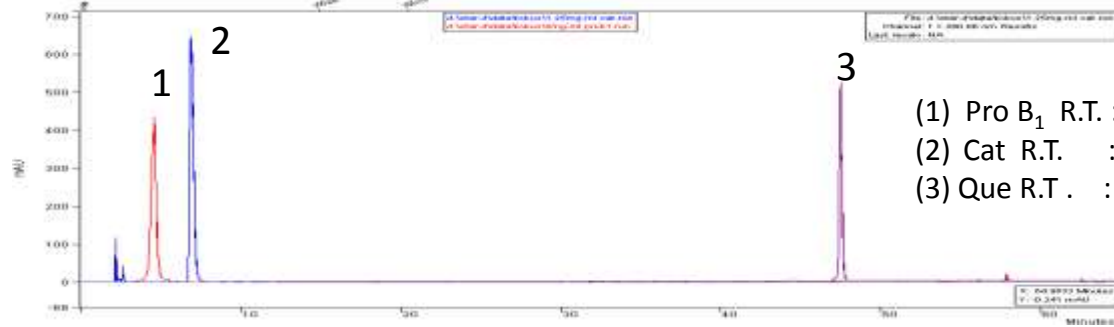




# HHP Effects on total phenolics, major polyphenols (Procyanidin B<sub>1</sub>, catechin, quercetin), antioxidant activity, microbial quality in huckleberry ice-cream

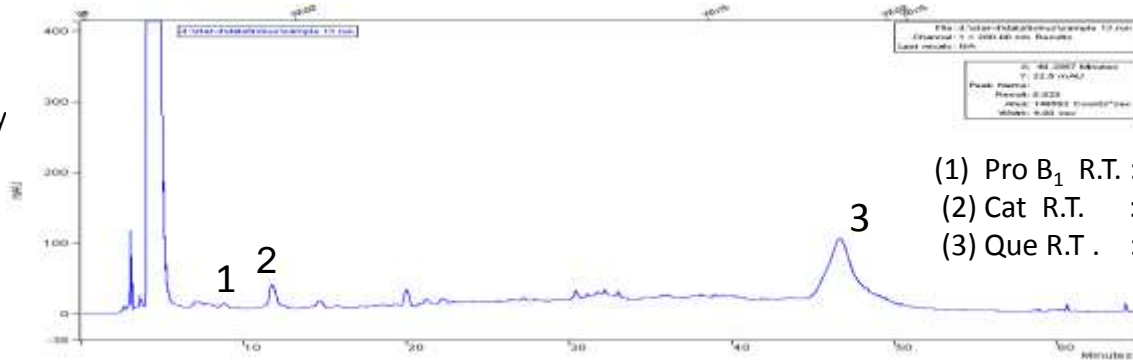
✓ In HHP treated huckleberry ice-creams, antioxidant activity, total phenolic levels increased (due to extraction capability rised). Especially, quercetin levels highly increased and microbial stability was highly preserved in HHP treated samples

Std.  
Chromatogram



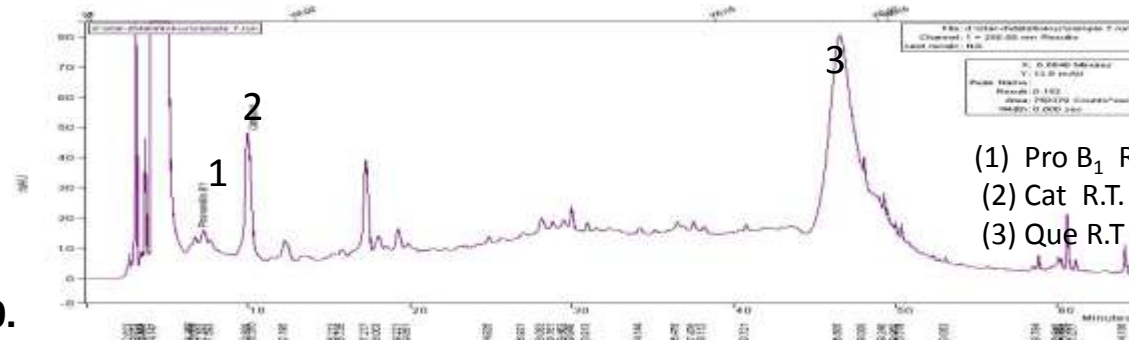
- (1) Pro B<sub>1</sub> R.T. : 7.57 min
- (2) Cat R.T. : 10.32 min
- (3) Que R.T. : 47.34 min

with Huckleberry  
ingredient  
control



- (1) Pro B<sub>1</sub> R.T. : 7.63 min
- (2) Cat R.T. : 10.38 min
- (3) Que R.T. : 47.34 min

with Huckleberry  
ingredient  
HHP-treated



- (1) Pro B<sub>1</sub> R.T. : 7.58 min
- (2) Cat R.T. : 10.32 min
- (3) Que R.T. : 47.33 min





It was determined that High Pressure Extraction (HPE) has tremendous potential for use in flavonoid extraction (Material: Litchi (*Litchi chinensis* Sonn.) fruit pericarp (LFP)).

The Litchi is the sole member of the genus Litchi in the soapberry family Sapindaceae. It is a tropical fruit tree. It is a fragranced fruit with a sweet taste.



✓ After 30 min of HPE of Litchi (*Litchi chinensis* Sonn.) fruit pericarp (LFP), the extract yield, total phenolic level, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), and superoxide anion scavenging ability were determined.



✓ The extraction yield by treatments of 400 MPa HPE for 30 min was 30%, while that by conventional extraction (CE, control) was 1.83%. There was no significant difference in the total phenolic content (as mg/g DW) among the two extraction methods (HPE and CE).

✓ It was found that the DPPH radical scavenging activity obtained by HPE (400 MPa) was the highest (74%), while that of ascorbic acid was the lowest (44%), when using a 10 mg/mL concentration.

**Table 3. The quantification of individual flavonoids from Litchi Fruit Pericarp Tissues By conventional extraction, ultrasonic extraction and high pressured-extraction**

Flavonoids (mg/g DW)*	Extraction Methods			
	CE	UE	HPE at 200 MPa	HPE at 400 MPa
Epicatechin	0.0414 ± 0.001	0.16 ± 0.04	0.32 ± 0.002	0.348 ± 0.06
Epicatechin gallate	0.0121 ± 0.003	0.06 ± 0.01	0.019 ± 0.04	0.2527 ± 0.04
Catechin	0.0002 ± 0.0	0.0020 ± 0.0005	0.0016 ± 0.001	0.0160 ± 0.07
Procyanidin B <sub>2</sub>	0.0175 ± 0.0003	0.0731 ± 0.0011	0.14 ± 0.03	0.1346 ± 0.03
Total flavonoids	0.0712 ± 0.004	0.2951 ± 0.051	0.6516 ± 0.07	0.7513 ± 0.2

Values reported are means of triplicate determinations ( $n=3$ ) ± SD ; DW\* = dry weight; CE= conventional extraction; UE= ultrasonic extraction; HPE=high-pressure extraction.

g.

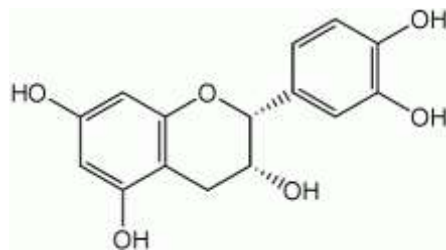
*This table was adapted from Prasad et.al.,2009 at Journal of Food Process Engineering*



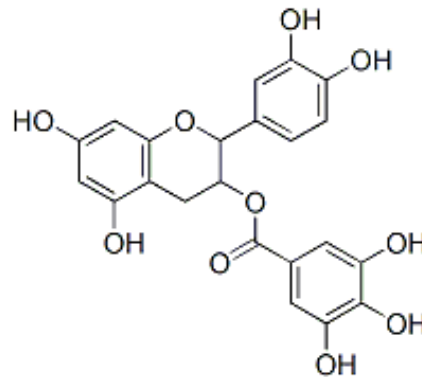
✓ Additionally, HPE could provide a more effective alternative than CE, because HPE requires less organic solvents and a shorter extraction time.

✓ Table 3 describes that the quantification of the individual flavonoids epicatechin (EC), epicatechin gallate (ECG), catechin (C), and procyanidin B<sub>2</sub> and total flavonoids from LFP tissues by conventional extraction (CE), ultrasonic extraction (UE), and HPE.

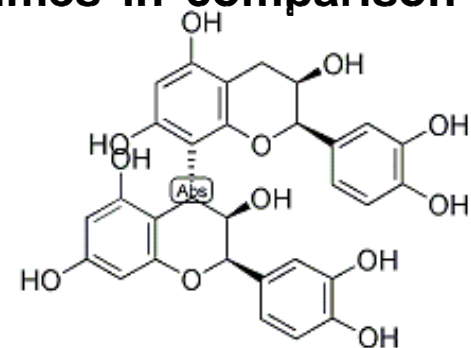
✓ EC and ECG were identified and quantified as the major flavonoids, while C and procyanidin B<sub>2</sub> were identified as the minor compounds. The total flavonoid content detected was 0.65, 0.75, 0.29 and 0.07 mg/g dry weight by HPE at 200 and 400 MPs, UE, and CE, respectively. HPE increased the flavonoid extraction yield up to 2.6 times in comparison with UE, and up to 10 times compared with CE.



Epicatechin (EC)



Epicatechin Gallate (ECG)



procyanidin B<sub>2</sub>

**Table 5.** A comparison of scavenging activities of superoxide anion radical and DPPH radical of HPE and CE extracts from longan fruit pericarp

	Superoxide scavenging activity (%)		DPPH scavenging activity (%)	
	50 $\mu$ g/ml	100 $\mu$ g/ml	50 $\mu$ g/ml	100 $\mu$ g/ml
<b>CE extract</b>	<b>48.6 <math>\pm</math> 2.9a</b>	<b>56.6 <math>\pm</math> 2.3b</b>	<b>50.1 <math>\pm</math> 0.2c</b>	<b>76.6 <math>\pm</math> 0.5b</b>
<b>HPE extract</b>	<b>50.4 <math>\pm</math> 0.9a</b>	<b>61.6 <math>\pm</math> 1.1a</b>	<b>75 <math>\pm</math> 0.2 b</b>	<b>77.7 <math>\pm</math> 0.2a</b>
<b>Ascorbic acid*</b>	<b>30.7 <math>\pm</math> 2.7b</b>	<b>42 <math>\pm</math> 2c</b>	<b>80 <math>\pm</math> 2 a</b>	<b>80.4 <math>\pm</math> 0.6a</b>
<b>BHT*</b>	<b>20 <math>\pm</math> 3.5c</b>	<b>23 <math>\pm</math> 1.7d</b>	<b>77.4 <math>\pm</math> 2 a</b>	<b>80 <math>\pm</math> 1a</b>

Conventional extraction (CE): 50% ethanol, 1:50 (w/v) solid/liquid ratio and 12 h of extraction time at 30 °C and high pressure extraction (HPE): 50% ethanol, 1:50 (w/v) solid/liquid ratio, 500 MPa pressure and 2.5 min of extraction time at 30 °C. For each treatment means within a column followed by different letters were significantly different at the 5% level. \*Positive controls





## ***DEMAND:***

***For highest quality smoothy products convenient,  
clean label,  
minimally processed,  
fresh-like flavour, taste and appearance  
good color stability***

## **Smoothy Ingredients**

**fruit (or less commonly vegetables),  
fruit juice,  
ice,  
yoghurt  
milk**

**Effect of HHP processing on fruit  
smoothy quality**



**Antioxidant activity and phenolic content of cranberry fruit smoothies was determined after the HHP processing (450 MPa/10 min) and during storage at 4 °C.**



**Mean values for redness ( $a^*$ ) showed an increase ( $p < 0.001$ ) in HHP processed smoothies compared to fresh.**

***HHP processing could help retain antioxidants in fruit smoothies offering a unique selling point for processors.***

## Effect of HHP processing on bluberry purees quality



**In HHP and thermally treated samples, the number of survivors of moulds and yeasts remained unchanged and below the detection limit ( $<10^1$  CFU/g) during the whole storage period (60 d), while untreated samples reached  $4.3 \pm 2.5$  log CFU/g after 60 d of storage.**

**The highest antocyanin extractability was found in those purees treated at the lowest high-pressure treatment intensity and holding time (450 MPa/5min), but at the end of the storage (day 60), no differences in individual or total carotenoid levels were found between the purees.**

- ✓ **HPP at 600 MPa/10 min showed the highest polyphenols content after the treatment and during the storage**
- ✓ **HPP is very effective for the reduction of moulds and yeasts counts. Based on the microbial inactivation, HPP can be an alternative for the conventional pasteurization.**

## Effects of HHP on on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps



- ✓ Total phenols increased at 500 or 600 MPa, the individual phenolic compounds and total phenols decreased at 400 MPa
- ✓ The monomeric anthocyanins, polymeric color and redness ( $a^*$ ) exhibited no change.
- ✓ HHP induced a decrease in lightness ( $L^*$ ) and an increase in yellowness ( $b^*$ ) at 400 MPa,

$\beta$ -Glucosidase was activated by 4.7–16.6% at 400 MPa 5–25 min<sup>-1</sup> and inactivated by 8.0–41.4% at 500 or 600 MPa. Polyphenol oxidase (PPO), peroxidase (POD) were inactivated at all pressures, the largest reduction in activity being 41.4%, 51.5% and 74.6%, respectively.

# **HHP Effects on Uncommon Mycotoxigenics**

Mycotoxin citrinin (CIT) is a toxic secondary metabolite, isolated from filamentous fungus *Penicillium citrinum* and is also produced by other species of *Penicillium*, *Aspergillus* and *Monascus*

Due to antibacterial effects of citrinin, it was investigated as an antibiotic but relative toxicity studies showed that citrinin acting in animals as a nephrotoxin damaged the proximal tubules of the kidney and was implicated as a causative agent in human endemic Balkan nephropathy



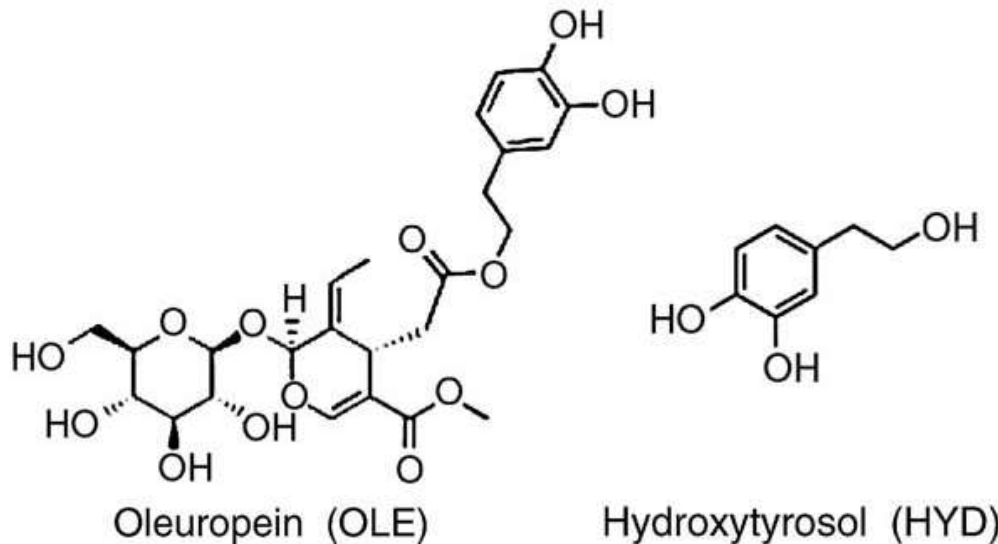
**Citrinin**  
**Mycotoxin**

((Betina, 1989), Frank, 1992)



# HHP Effects on total phenolics, major polyphenols (hydroxytyrosol, oleuropein), antioxidant activity, microbial quality and mycotoxin citrinin and OTA content in black and green table olive fruits

- ✓ The total phenolics of table olives increased (2.1–2.5)-fold after HPP (as mg gallic acid equivalent/100 g).
- ✓ Phenolic hydroxytyrosol in olives increased on average (0.8 – 2.0)-fold, whereas oleuropein decreased on average (1 – 1.2)-fold after HPP (as mg/kg dwt).
- ✓ Antioxidant activity values varied from 17.238 – 29.344 mmol Fe<sup>2+</sup>/100 g for control samples, and 18.579 – 32.998 mmol Fe<sup>2+</sup>/100 g for HPP-treated samples.



Major olive fruit phenolics

**Olives**



**Table 6.** Major phenolics hydroxytyrosol (HYD), oleuropein (OLE), and total phenolic profiles of control and HHP-treated black table olives

Olive sample	Major phenolics (as mg/kg dwt)				Total phenolics (as mg GA/100 g dwt)	
	Hydroxytyrosol (HYD) control	Hydroxytyrosol HYD HHP-treated	Oleuropein (OLE) control	Oleuropein (OLE) HHP-treated	TP control	TP HHP-treated
Vakum F-XL	8200.6 ± 2.93	11386.9 ± 1.87	1036.64 ± 0.56	750.98 ± 1.05	1788.2 ± 6.07	4073.2 ± 1.00
Vakum F-L	9486.1 ± 2.03	12772.9 ± 0.17	1272.1 ± 0.09	914.0 ± 0.00	1853.7 ± 3.56	4895.1 ± 3.33
F-Tin	5420.6 ± 0.78	6201.1 ± 7.05	2441.2 ± 1.90	1148.8 ± 1.76	1455.2 ± 2.55	3117.7 ± 0.97
Dock out-F	6662.4 ± 0.50	7002.7 ± 3.37	1758.0 ± 0.88	1084.8 ± 2.33	1677.1 ± 0.99	3551.0 ± 1.54
Washed/selected-F	6820.2 ± 1.23	8015.9 ± 0.05	1802.3 ± 0.44	1021.2 ± 0.56	1694.6 ± 1.58	3846.2 ± 1.90
Pastör.out-F	5498.0 ± 1.07	7291.7 ± 1.74	1739.2 ± 2.11	1000.1 ± 0.06	1416.5 ± 3.00	3677.5 ± 0.11
Vakum U.Özel	7101.6 ± 0.73	8005.4 ± 1.06	1355.6 ± 1.08	957.0 ± 0.18	1711.7 ± 2.82	3267.6 ± 2.72
Light U.Gemlik.Type	3937.3 ± 1.37	4660.1 ± 5.08	778.6 ± 0.06	518.5 ± 0.95	1292.2 ± 0.71	2669.9 ± 1.13
Light U.Aegean.Type	4522.5 ± 3.86	5240.6 ± 1.77	794.3 ± 0.19	547.2 ± 2.12	1570.8 ± 4.11	3141.5 ± 4.72
Vakum U.Jumbo	7708.3 ± 4.06	8115.0 ± 0.09	2121.6 ± 0.75	1910.1 ± 1.05	1792.6 ± 1.74	3518.1 ± 0.85
U-Sele	7277.5 ± 3.15	7965.3 ± 0.17	1962.5 ± 1.23	1623.3 ± 0.79	1432.5 ± 0.97	2542.3 ± 1.07
U-Tin	5007.2 ± 0.99	6487.5 ± 1.43	2155.5 ± 3.00	1362.6 ± 3.02	1380.8 ± 5.04	2852.5 ± 2.08

Data are expressed as an average of two determinations corresponding to the duplicate extraction of each injected duplicate. Mean concentrations with ± standard deviations ( $n=2$ ).

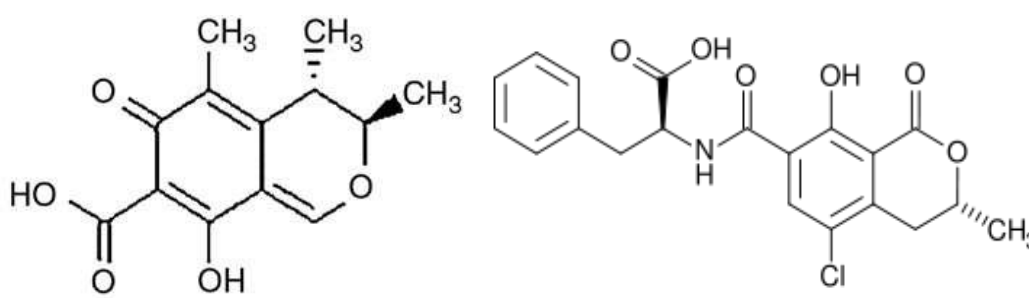
**Company: FORA OLIVE**

*Tokuşoğlu, Alpas & Bozoğlu, 2010 (Innovative Food Sci and Emerging Technologies)*

**Table 7.** The antioxidant activity (as FRAP values mmol Fe<sup>II</sup>/100g) values in selected table olives

Olive sample	Antioxidant activity (FRAP values mmol Fe <sup>2+</sup> /100 g)	
	Control	HHP
Vakum F-XL	25.320 ± 1.21	27.320 ± 0.72
Vakum F-L	29.344 ± 0.96	32.998 ± 1.03
F-Tin	20.215 ± 2.05	22.215 ± 0.11
Dock out-F	21.013 ± 0.15	22.532 ± 0.42
Washed/selected-F	22.505 ± 1.45	23.237 ± 0.85
Pastör.out-F	20.233 ± 0.50	21.965 ± 0.15
Vakum U.Özel	23.262 ± 1.78	25.488 ± 0.66
Light U.Gemlik.Type	17.238 ± 0.47	18.579 ± 0.53
Light U.Aegean.Type	19.056 ± 0.55	21.290 ± 0.5
Vakum U.Jumbo	24.289 ± 0.30	25.210 ± 0.58
U-Sele	20.402 ± 0.52	20.996 ± 0.07
U-Tin	19.533 ± 0.25	22.285 ± 0.10

Data are expressed as an average of two determinations corresponding to the duplicate reading. Mean concentrations with ± standard deviations ( $n = 2$ ).



**Mycotoxin Citrinin (CIT)**

**Mycotoxin Ochratoxin A (OTA)**

**Olive Fruit Mycotoxins**

✓ In the HPP applied olives, total mold was reduced 90% at 25 °C, and it was reduced 100% at 4 °C . Total Aerobic-Mesophilic Bacteria load was reduced 35 – 76% at 35 ± 2 °C.

✓ Citrinin load was reduced 64 – 100% at 35 ± 2 °C. Especially, 1 ppb and less CIT contamination in table olives degraded as 100%.

**Table 8. CIT levels in control and HHP-treated olives**

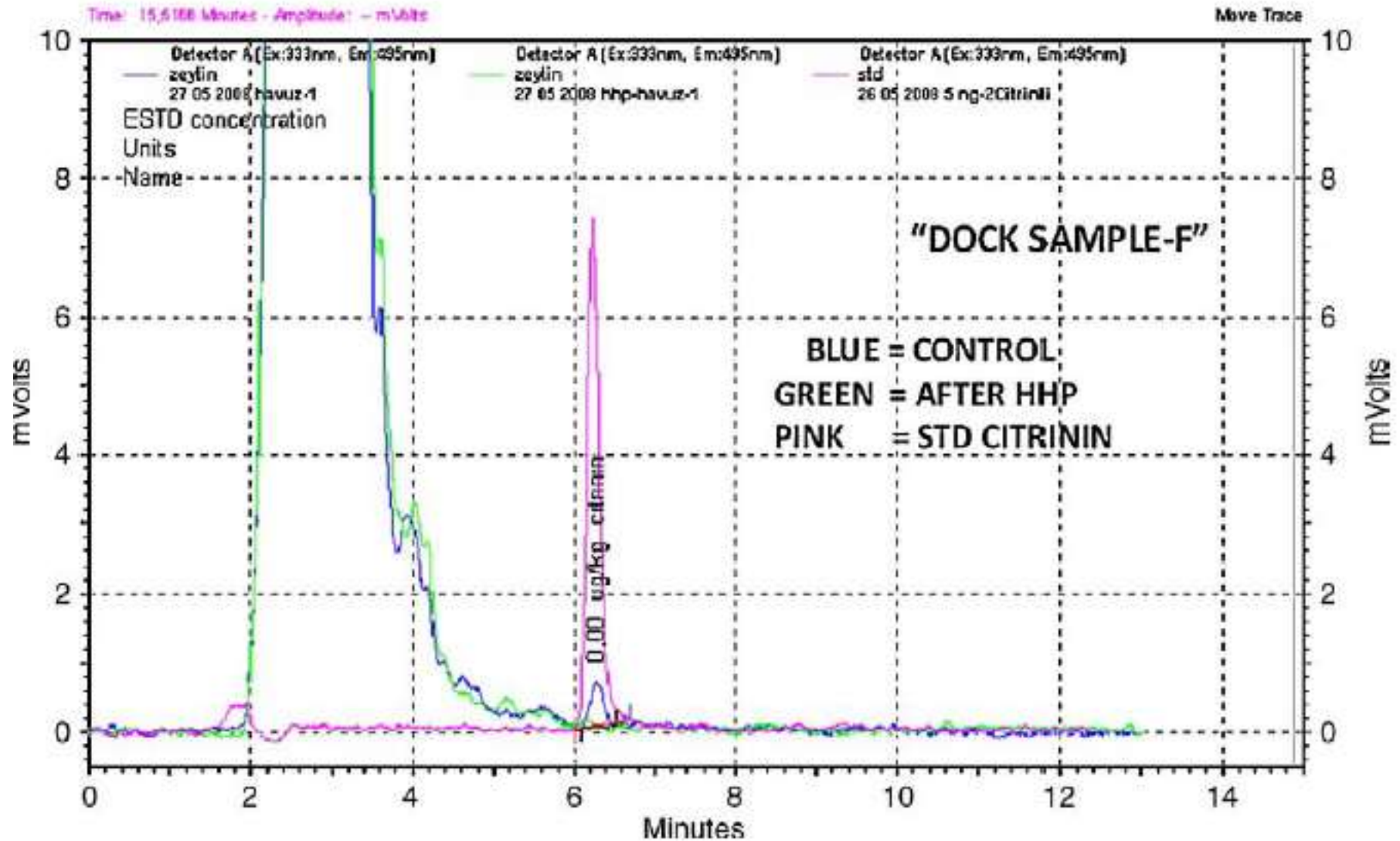
Citrinin data (as µg/kg dwt)

	Vakum F-XL	Vakum F-L	F-Tin	Light U.Gemlik Type	Light U.Aegean Type	Vakum U.Jumbo	Vakum U.Özel	U-Tin	U-Sele	Dock out-F	Pastör. out-F	Washed/ selected-F	Dock out-F/ liquid	Pastör.out-F/ liquid
Control	0.11 ± 0.01	ND	<0.10 ± 0.01	ND	ND	0.14 ± 0.01	ND	<0.10 ± 0.01	0.12 ± 0.02	0.53 ± 0.05	ND	<0.10 ± 0.01	2.26 ± 0.11	1.71 ± 0.02
HHP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.19 ± 0.05	0.58 ± 0.02

Data are expressed as an average of two determinations corresponding to the duplicate extraction of each injected duplicate. Mean concentrations with ± standard deviations (n=2). Not detected = ND.

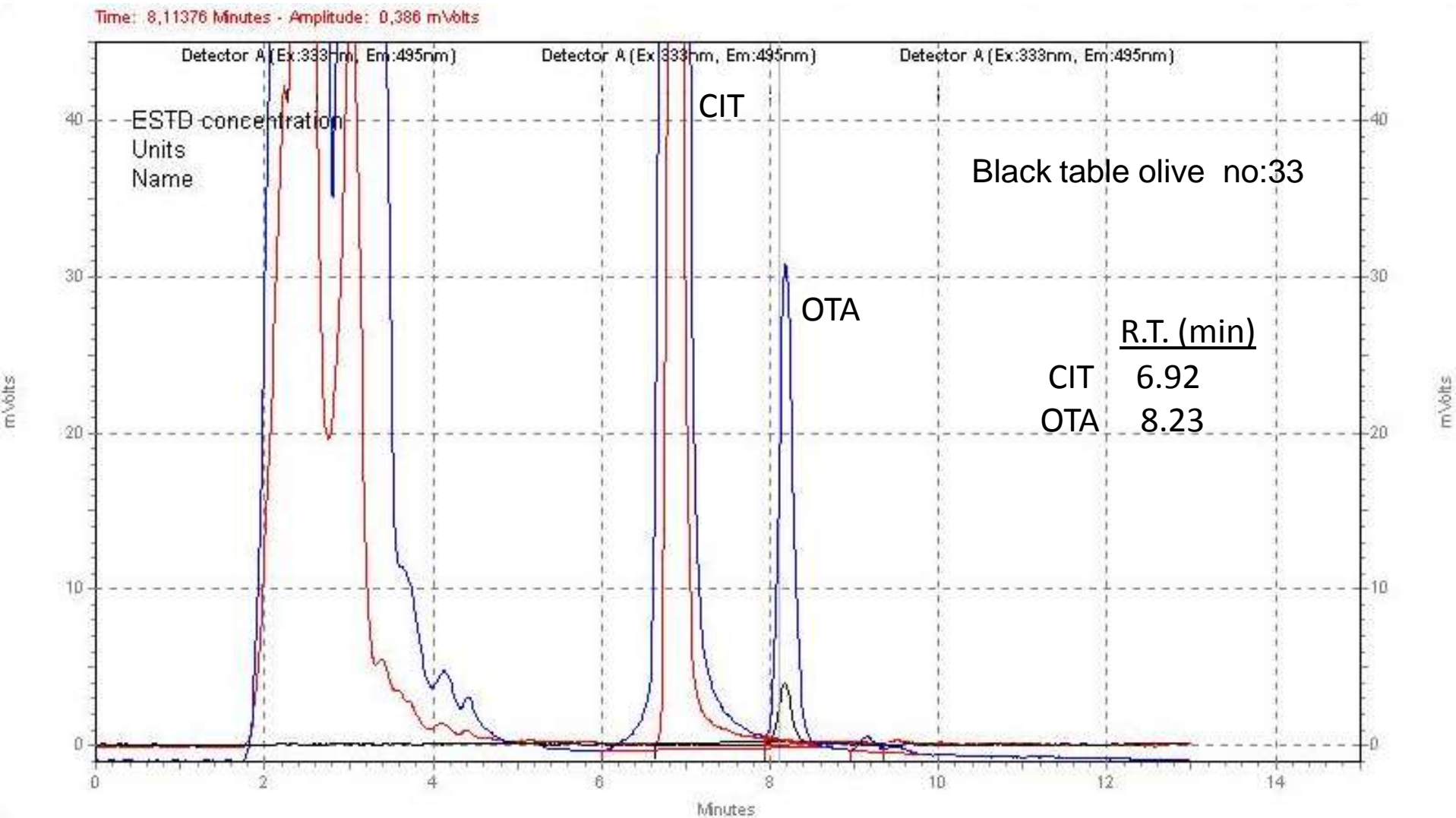
*Tokuşoğlu, Alpas & Bozoğlu, 2010 (Innovative Food Sci and Emerging Technologies);  
Tokuşoğlu & Bozoğlu, 2010 (Italian Journal of Food Sci)*

# HPLC Chromatogram of CIT occurrence in control and HHP-treated olive sample



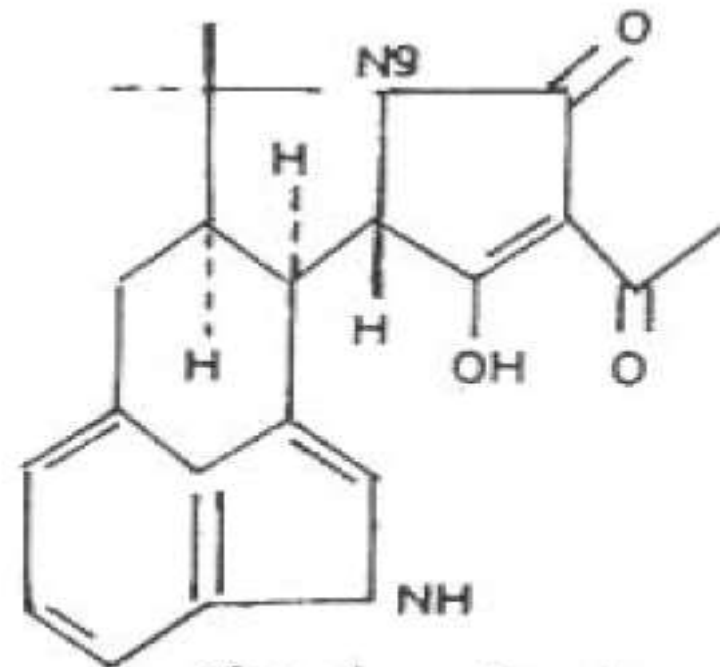
HPLC chromatogram of citrinin occurrence in control “dock-F” and HHP-treated “dock-F” olives and standard citrinin.

# HPLC Chromatogram of CIT & OTA in control and HHP-treated olive sample



**Besides AFs,  
some A. flavus strains  
together with strains  
belonging to the species  
Aspergillus tamarii, also  
included in the section Flavi,  
are reported to produce  
cyclopiazonic acid (CPA).  
CPA mycotoxin is a specific  
inhibitor of calcium-  
dependent ATPase, which is  
toxic to animals and humans**

**Mycotoxin  
Cyclopiazonic Acid**



Siklopiazonikasit

(Horn, 2007).

**In relation to the hygienic hazard and the economic incidence of cyclopiazonic acid contamination. It has hepatotoxicity, cancerogenic and neurotoxigenic**

**AD Level CPA : 200  $\mu\text{g}$  dir.**

**Daily 50 g consumption of cheese;**

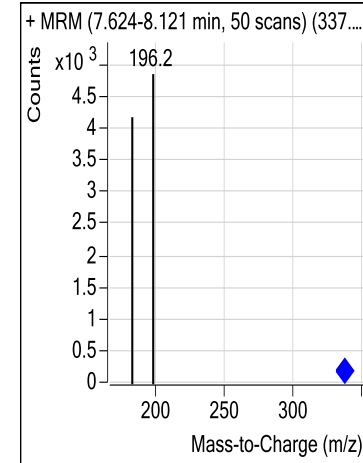
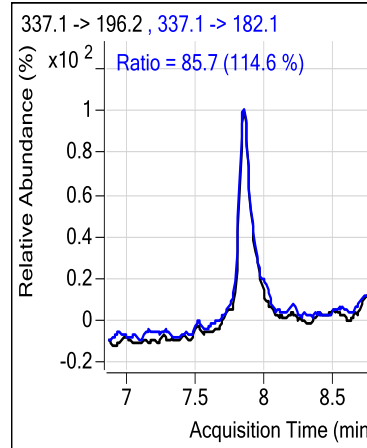
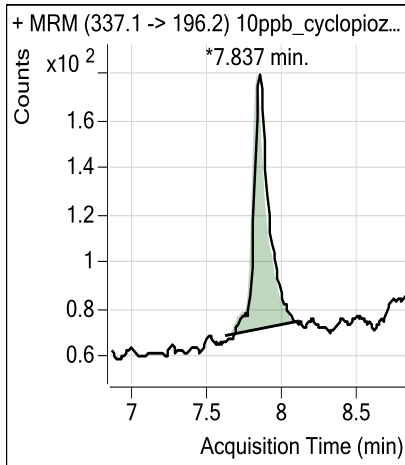
**CPA absorption: 4  $\mu\text{g}$  /g.**

**HHP applied to the manufactured cheeses in controlled conditions in company (*Ekici*)**

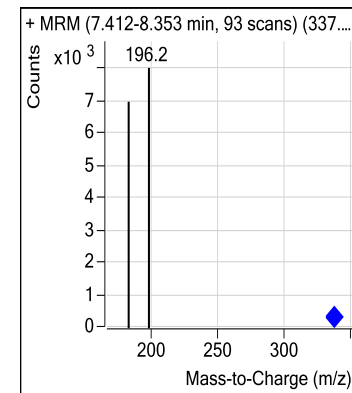
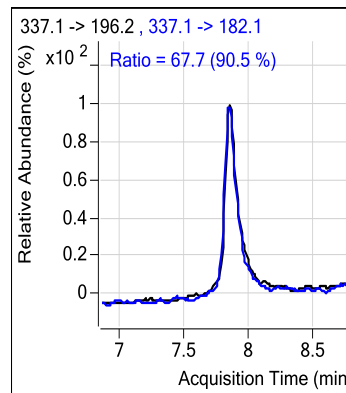
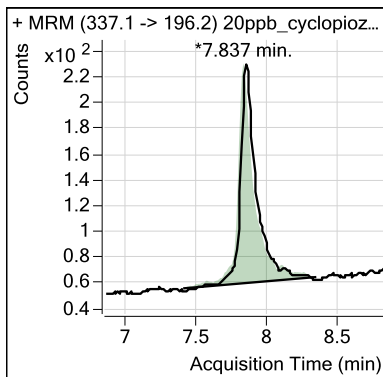
<b>Sonuçlar</b>				
		<b>RT</b>	<b>Concentr</b>	
10 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.837	9.540	ng/ml
20 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.837	19.966	ng/ml
50 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.867	51.836	ng/ml
100 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.867	100.347	ng/ml
200 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.847	199.404	ng/ml
2 ppb cyclopiozonic acid.d	Cyclopiozonic_acid	7.928	0.904	ng/ml



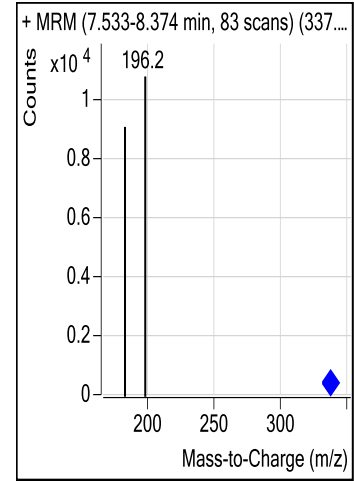
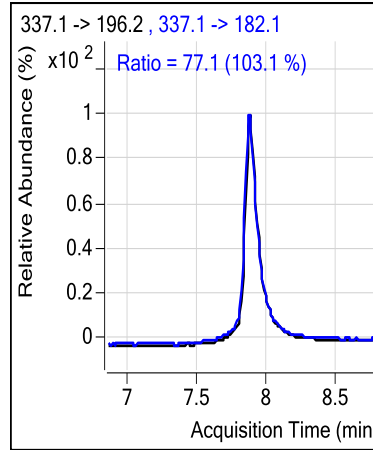
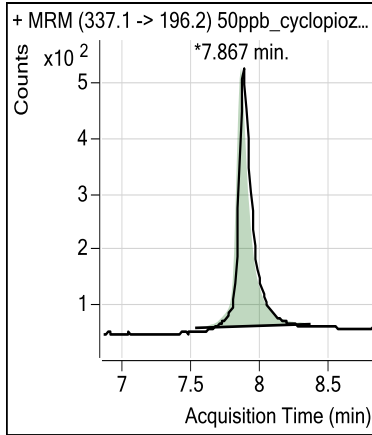
# 10 ppb



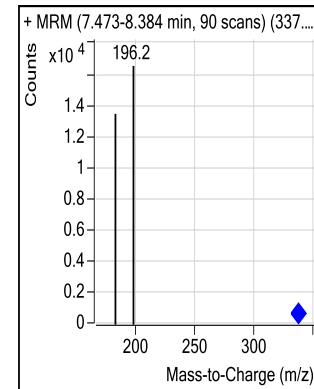
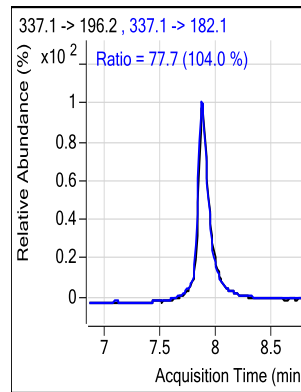
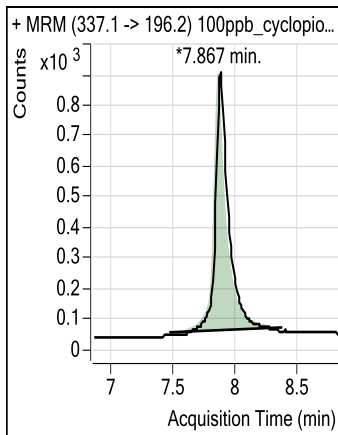
# 20 ppb



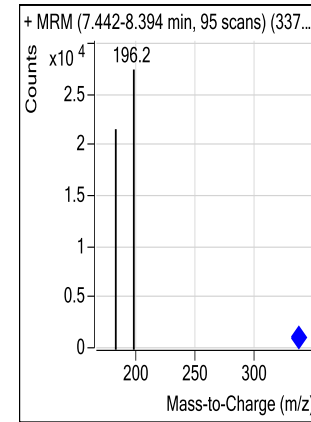
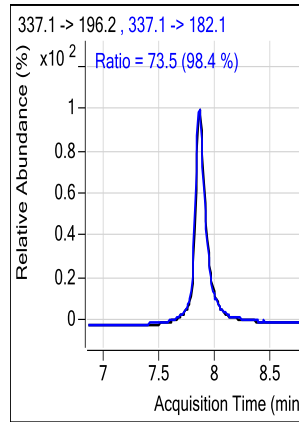
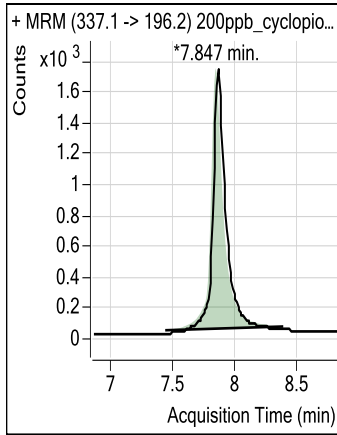
# 50 ppb



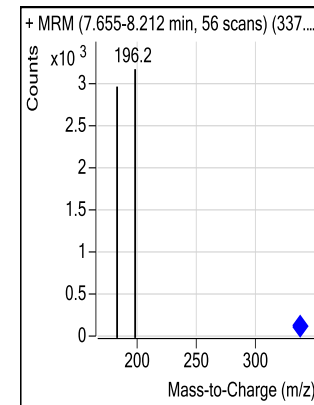
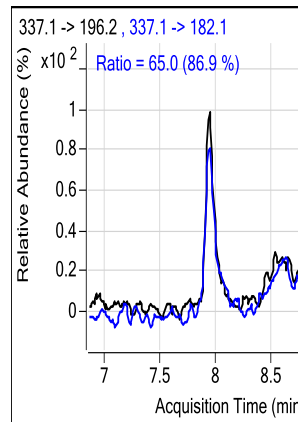
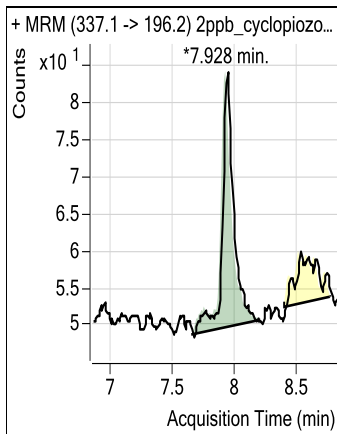
# 100 ppb



# 200 ppb



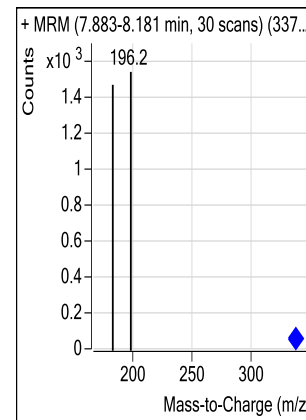
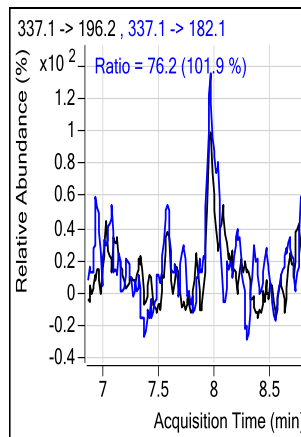
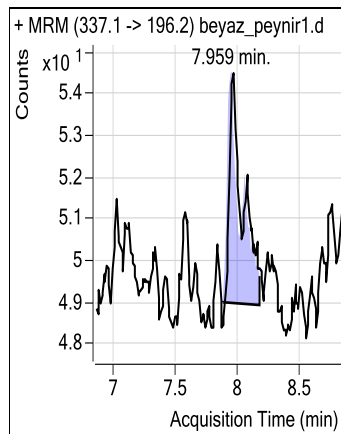
# 2 ppb



<b>RESULTS</b>	<b>HHP 400 MPA / 12 min after homogenization and pH11 process</b>			
		<b>RT</b>	<b>Concent</b>	ng/ml
White cheese 1	Cyclopiozonic _acid	7.888	5.2246	ng/ml
White cheese 2	Cyclopiozonic _acid	7.523	0.9315	ng/ml
HHP treated White cheese 1	Cyclopiozonic _acid	7.959	0.0000	ng/ml
HHP treated White cheese 2	Cyclopiozonic _acid	7.979	0.0000	ng/ml

# White Cheese

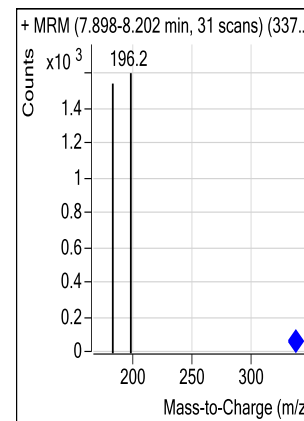
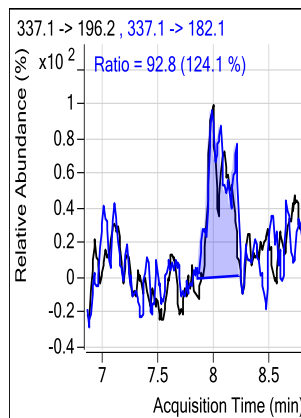
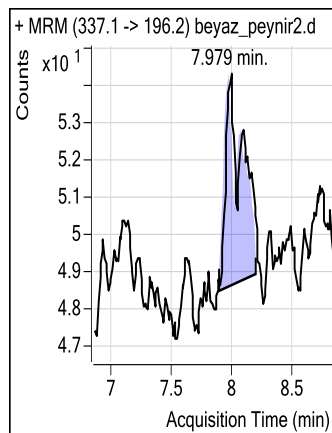
Beyaz peynir1.d



After HHP

400 MPA /  
12 min

Beyaz peynir2.d



Control 1

Control 2

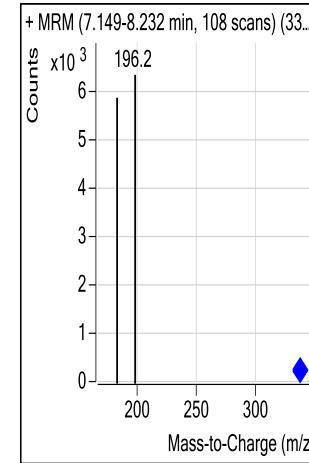
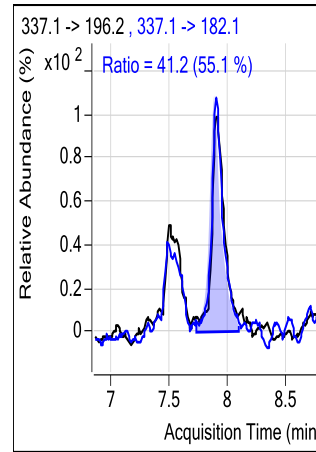
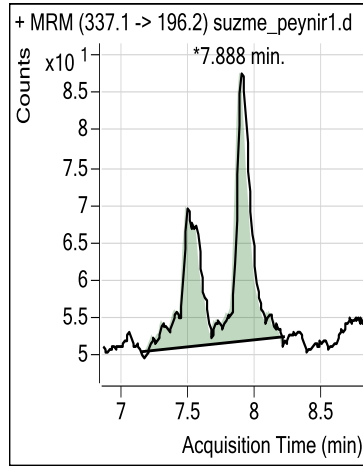
		RT	Concent	ng/ml
Suzme cheese 1	Cyclopiozonic _acid	7.888	4.2246	ng/ml
Suzme cheese 2	Cyclopiozonic _acid	7.523	0.8670	ng/ml
HHP treated Suzme cheese 1	Cyclopiozonic _acid	7.959	0.0000	ng/ml
HHP treated Suzme cheese 2	Cyclopiozonic _acid	7.979	0.0000	ng/ml

**After homogenization step of the cheese manufacturing, HHP 400 MPA / 12 min was applied**

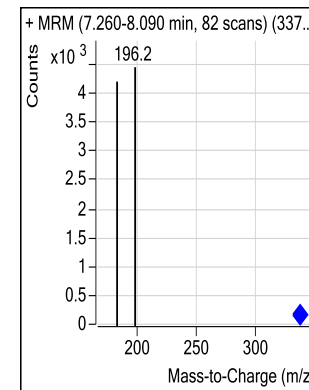
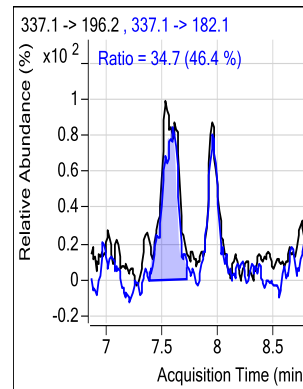
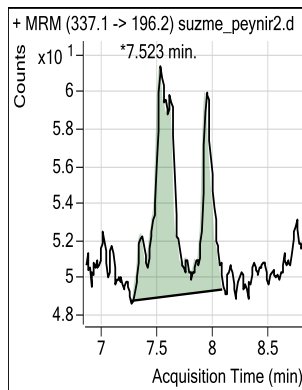
# Süzme Cheese

After HHP

400 MPA  
12 min



Süzme peynir 2.d



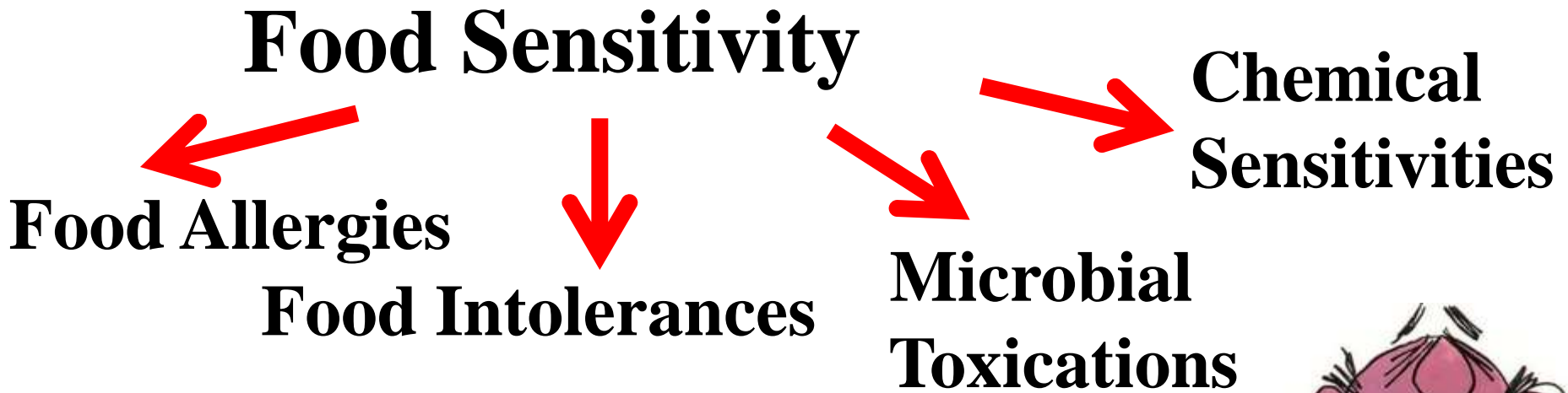
Control 1

Control 2

# **HHP Effects on Allergens**



**Food sensitivity is an adverse reaction to a food which other people can safely eat.**



# **FOOD ALLERGIES**

**Food allergy is an abnormal response to a food triggered by body's immune system. Foodborne allergic reactions can sometimes cause serious illness and death.**



**Distinguishing food allergy from other food sensitivities is the most important. Whereas food allergies are rare, food intolerances, which are the other classification of food sensitivities, are more prevalent.**

# Most Common Food Allergens

## Tree nuts

*almonds, brazil nuts, cashews, Hazelnuts (filberts), macadamia nuts, pecans, pine nuts (pignolias), pistachio nuts, walnuts*

## Peanuts

## Cow's milk

## Soy /Gluten

## Wheat

## Eggs

## Fish /Shellfish

## Sesame seeds

## Faba beans

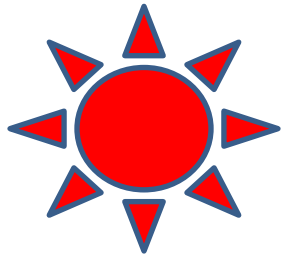
## Garlic /Onion /Mustard



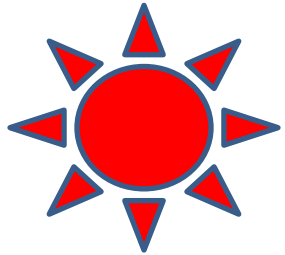


**Allergens are generally food proteins.**

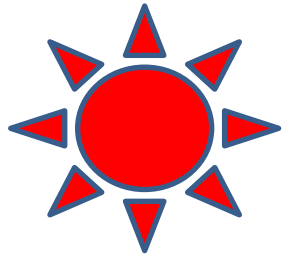
**Some consumers are genetically predisposed and their immune system is not able to differentiate the food protein from the virus or bacteria, thereby attacks occur.**



**1. Effect IgE**



**2. Effect Histamin**

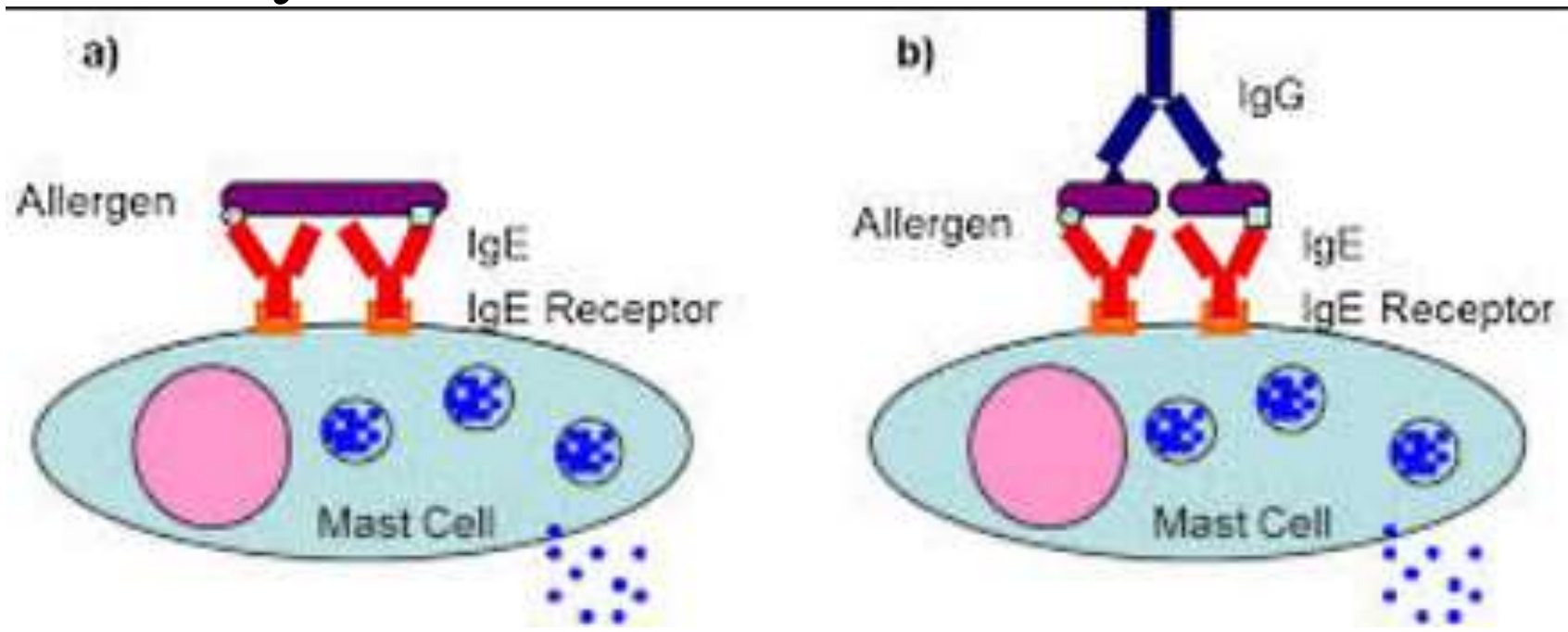


# 1. Effect

Some proteins or protein fragments are resistant to digestion and those that are not broken down in the digestive process are tagged **by the Immunoglobulin E (IgE)**. These tags trick the immune system into thinking that the protein is harmful.

It is stated that the IgE acts like a tag, sticking to molecules in food or pollen called allergens. If an allergic consumer eats a beleaguered (confined) food, IgE attaches to the allergens, setting off an allergic reaction in the body

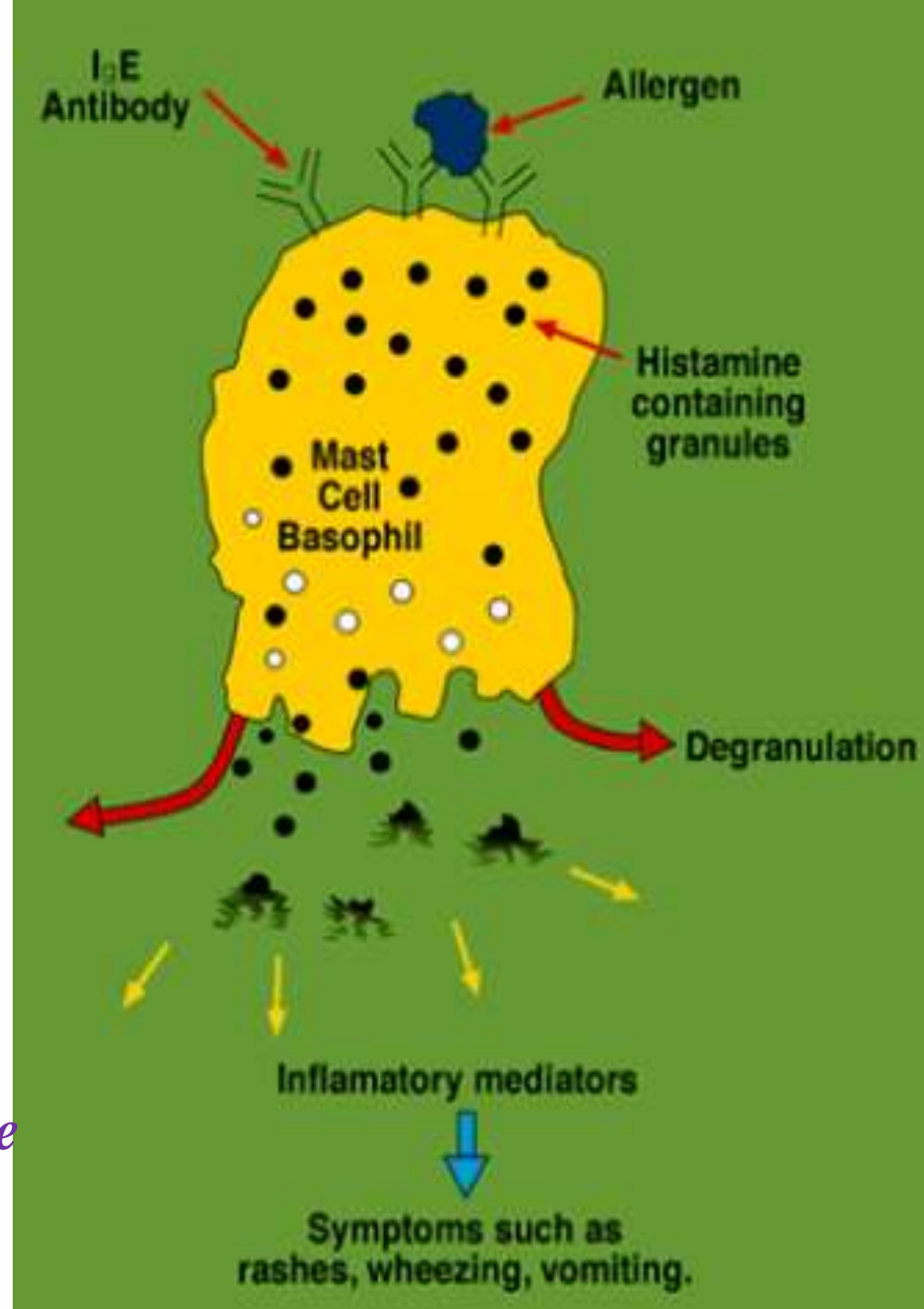
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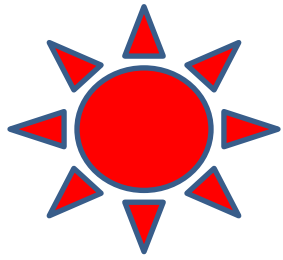




**Macrophage cells are known as scavenger cells in the immune system; these are particularly designed for removing damaging molecules from the body.**

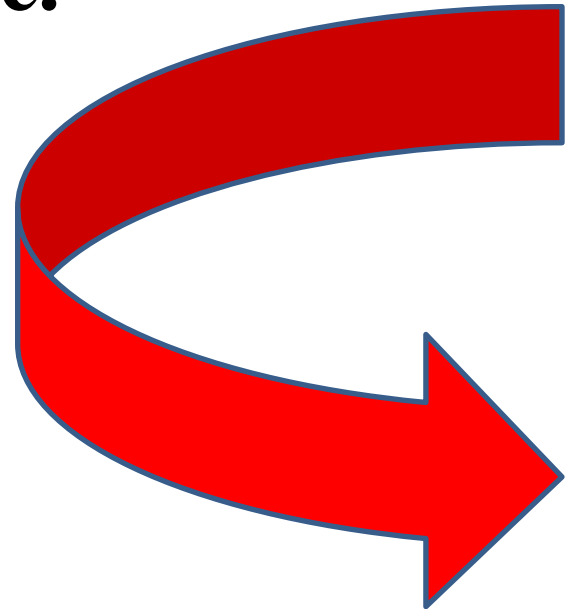
*The macrophages consume the molecule, taking it out of circulation and exterminating it; afterward the antibody binds to the dangerous molecule*





## 2. Effect

Another recognized effect that IgE induces the **histamine**, which can lead to the alterations. It can be seen in our bodies as symptoms, like nettle rash or wheezing, and these reactions can be ranged from mild to severe.



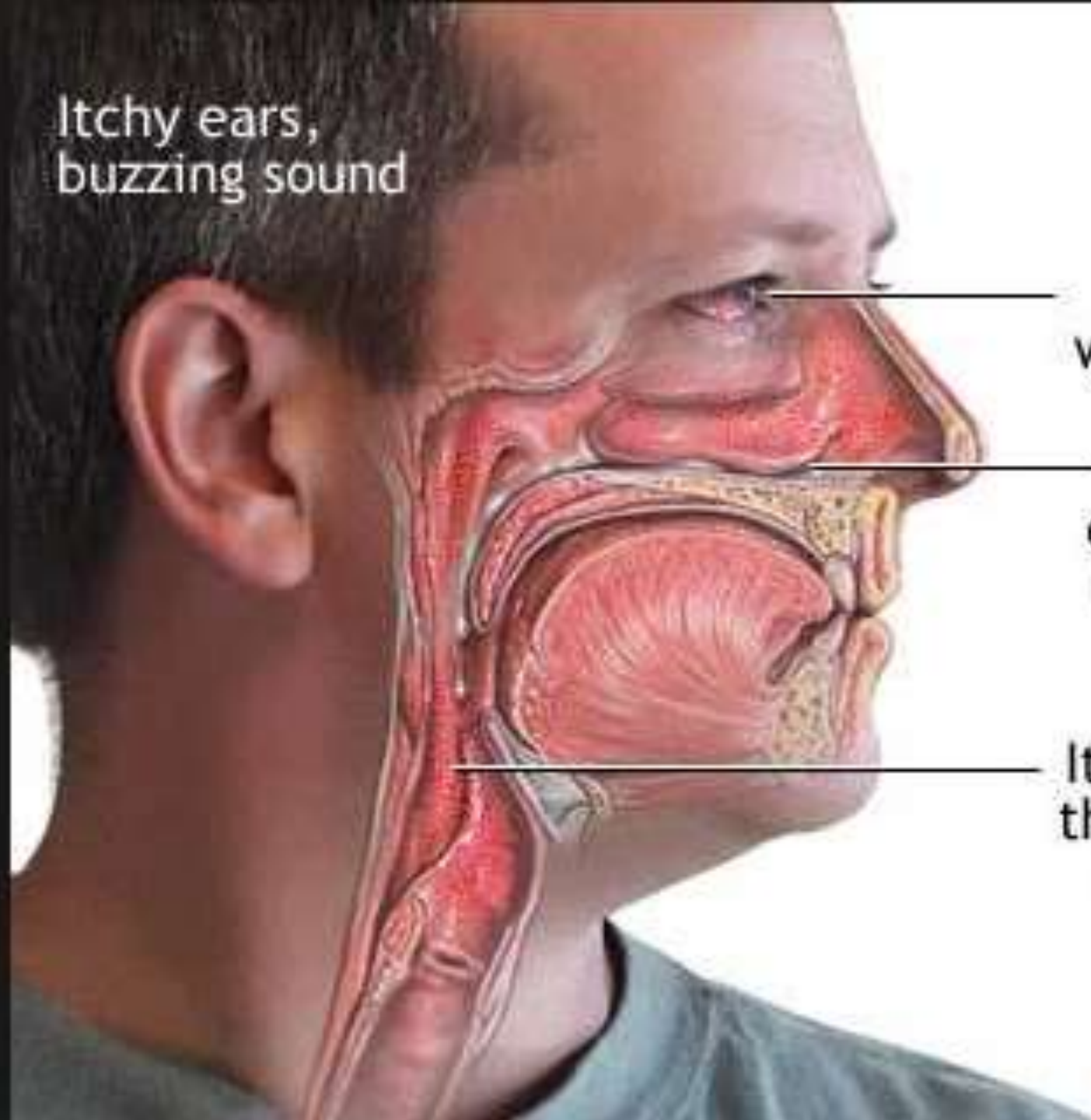


Itchy ears,  
buzzing sound






Red, itchy,  
watery eyes

Sneezing,  
congestion,  
runny nose

Itchy or sore  
throat, post-  
nasal drip,  
cough



*The above-mentioned symptoms are not seen so often with food allergies. When contacting a food, itching and swelling around the lips and mouth may occur and also*

-  **nausea,**
-  **cramping pains,**
-  **bloating,**
-  **vomiting**
-  **diarrhea**

*Food allergy symptoms vary from mild localized symptoms to severe anaphylaxis that, at times, may be fatal.*

# **Conventional Processing Effects on Food Allergenicity**

**Due to the ubiquitous presence of allergens in the food supply, reducing/elimination strategies of allergens in foods are important. Using food processing to reduce/eliminate allergenicity have been performed**

**It has been shown that food processing has important effects on the structural and allergenic properties of food allergens**

**It is stated that cupins allergens, such as Ara h 1 from peanut and Lipocalins such as  $\beta$ -Lg, and  $\alpha$ -lactalbumin from milk may partially denature by thermal processing.**



**The effects of thermal processing in these allergens are partial unfolding of proteins and aggregation to form networks as emulsifiers around lipid or gelled systems. Maillard modifications may also potentiate allergenicity by thermal processing**

# Type of Processing Behavior

# Effect of Thermal Processing

# Types of Food Allergen

Processing-labile allergens

Protein unfolding, modification by Maillard adducts in sugar-rich foods, modification by polyphenols

Bet v 1 homologues from fruits such as Mal d 1, Pru av 1

Partially-denatured allergens

Partial unfolding of proteins, aggregation to form networks as emulsifiers around lipid or gelled systems. Maillard modifications may potentiate allergenicity

Cupins allergens, such as Ara h 1 from peanut. Lipocalins such as  $\beta$ -Lg and  $\alpha$ -lactalbumin from milk;

Allergens able to refold

Proteins unfold to a limited extent during heating but can re-fold on cooling. Maillard modification may potentiate allergenicity

Prolamin superfamily members belonging to the ns LTP, and 2S albumin sub-families such as Mal d 3; tropomyosins and parvalbumins

Mobile rheomorphic proteins

Proteins do not adopt a rigid conformation but are very mobile and consequently do not denature following thermal treatment

Caseins, seed storage prolamins of wheat (gluten), ovomucoid



# High Pressure Processing on Allergenicity and Allergen Compounds

**Thermal treatments, enzymatic treatments, and other conventional methods have generally been used for eliminating food allergenicity, some treatments gives;**

**degradation of processed food characteristics, (deterioration in the flavor and taste; (bitterness etc., unpleasant odor).**

***Besides, the enzymatic treatment applications to foods give a high level of protein; this situation is not practicable.***

**High-pressure (HP) processing treatments are novel-processing techniques that have the potential to alleviate the need for thermal processing of foods. High pressure (400–700 MPa) combined with temperatures around room temperature (5–40°C).**

**It is stated that treatments offer an alternative to high-temperature pasteurization, or chemical preservation and fresh-like properties of foods are preserved**

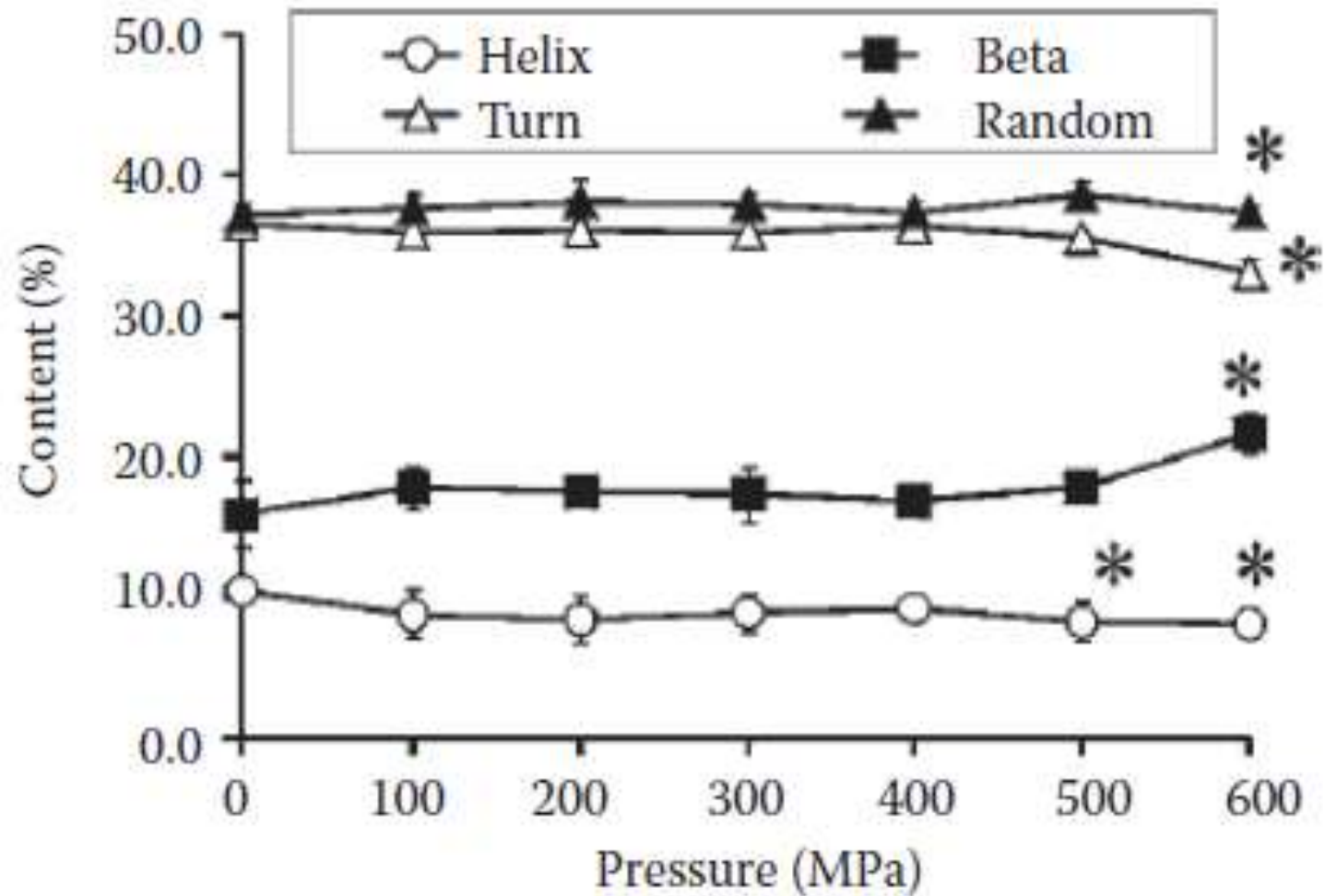
**Currently, limited studies have been performed on the HP processing effects on the structure of known allergens and the elimination of allergen compounds in foods.**

It was stated that the effects of a HP treatment (100-600 MPa/5–7 °C/5 min) on the IgE-specific binding activity and structural changes to bovine gamma globulin (BGG), a beef allergen, were investigated and then the allergenicity of pressure-treated BGG was evaluated. It was found that the IgEspecific binding activity and allergenicity of BGG were (89%) decreased by the HP treatment.

**For Meats Allergens**

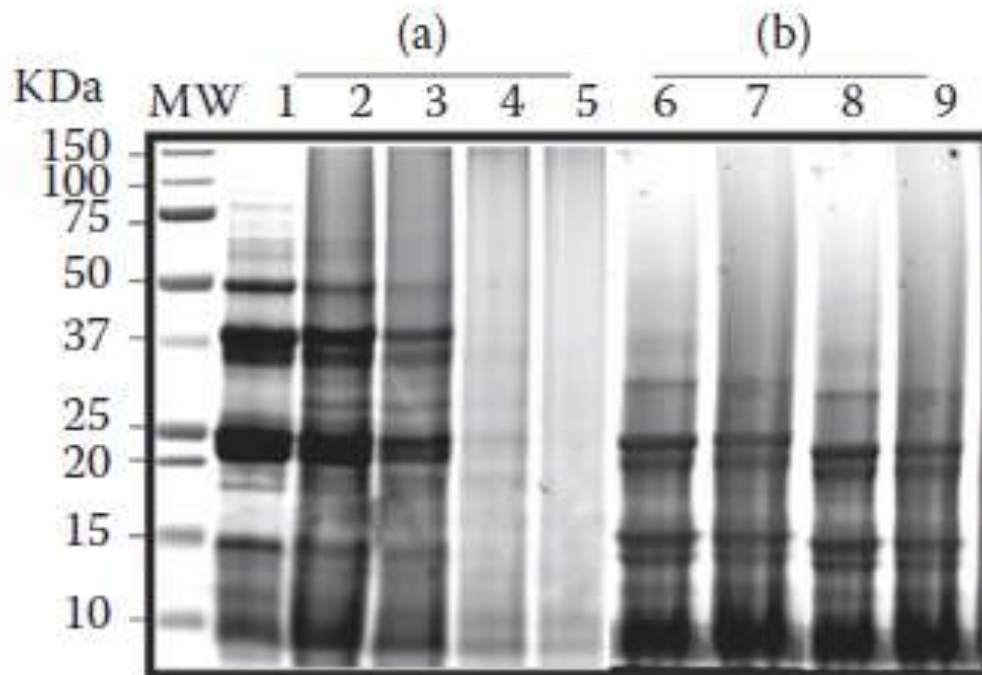


# HHP Effects on the Secondary Structure of BGG



**It was evaluated the effects of autoclaving (AC) and high hydrostatic pressure (HHP) processing effects on the allergenic characteristics of proteins from hazelnuts and Ig proteins decreased as 93%**

*HHP 400 MPa , 15 min*



- (1) Raw flour hazelnut
- (2) AC 121°C 15 min.
- (3) AC 121°C 30 min.
- (4) AC 138°C 15 min.
- (5) AC 138°C 30 min.
- (6) HHP 300 Mba
- (7) HHP 400 Mba
- (8) HHP 500 Mba
- (9) HHP 600 Mba

**For  
Peanut  
Allergens**

Hazelnut flour samples analyzed by SDS-PAGE

It is known that egg proteins are responsible for one of the most common forms of food allergy, especially in children, and one of the major allergens is ovalbumin (OVA).



**For  
Egg Allergens**

**Characterization of the  
hydrolysates and  
peptide identification was  
performed by RP-HPLC-  
Tandem Mass Spect  
(RP-HPLC-MS/MS).**

*The treatment with pepsin at 400 MPa/5 min/, all of the intact protein (100%) was removed in minutes, leading to the production of hydrolysates with lower antigenicity than those produced in hours at atmospheric pressure*

# **For Milk Allergens**



**It was stated that cow's milk protein allergy (CMPA) was the most prevalent allergy for infants or young children, with an incidence of about 2% to 7.5% in population-based studies in different countries**



■ It was reported that  $\beta$ -lactoglobulin ( $\beta$ -LG),  $\alpha$ -lactalbumin ( $\alpha$ -LA), and caseins are the main allergens in cow's milk; other proteins, such as bovine serum albumin (BSA) and even lactoferrin (present in trace amounts) are also potential allergens.

■ It is known that the differences between cow's milk protein and human milk protein composition may be one of the reasons that induce cow's milk allergy in infants



$\beta$ -lactoglobulin can be efficiently hydrolyzed by various enzymes under high pressure. It was shown that the hydrolysates obtained via the enzymatic treatment of  $\beta$ -LG under high pressure exhibited reduced antigenicity and IgE binding.

**It was reported that HP treatment (100–300 MPa) enhanced dairy whey protein hydrolysis and reduced the residual antigenicity of the hydrolysates, depending upon the choice of enzymes including trypsin, chymotrypsin, and pepsin**



**Rice is the main and most important food taken every day in Eastern Asia.**

**The prevalence of IgE-mediated rice allergy is about 10% in atopic subjects in Japan, whereas the frequency of rice allergic reactions is much lower in Europe and in the US.**

*It was stated that the 16-kDa rice allergen, RA17, belonging to the alpha-amylase/trypsin inhibitor family was isolated from rice seed and structurally characterized by identifying cystine-containing peptides and predicting the secondary structure and hydrophobic regions.*

*Izumi et al. (1999)*

**It was stated that protein is released from rice grains during HP treatment. Polished rice grains were immersed in distilled water and pressurized at 100–400 MPa (opt.300 MPa/ 10 min), so a considerable amount of proteins (0.2–0.5 mg per gram of grains) were released.**



**The released proteins were identified as 16 kDa albumin, alpha-globulin, and 33 kDa globulin, which were known as major rice allergens after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analyses**

**It was concluded that these allergenic proteins content decreased by pressurization and almost completely disappeared from target foods by the pressurization in the presence of some proteolytic enzymes or some pretreatments.**

**HHP processing improved the reducing of allergenic structure and allergenicity of some foods. Further studies are needed for some allergenic proteins in various food matrices.**



**From a nutritional prospective, HPP is an excellent food processing technology which has the new potential to retain the bioactive constituents with health properties in fruits, cereals, and other foods. HPP-treated foods retain more of their fresh-like features and can be marketed at a premium over their thermally processed counterparts.**

*Many thanks...*

## Fruit and Cereal Bioactives: Sources, Chemistry, and Applications

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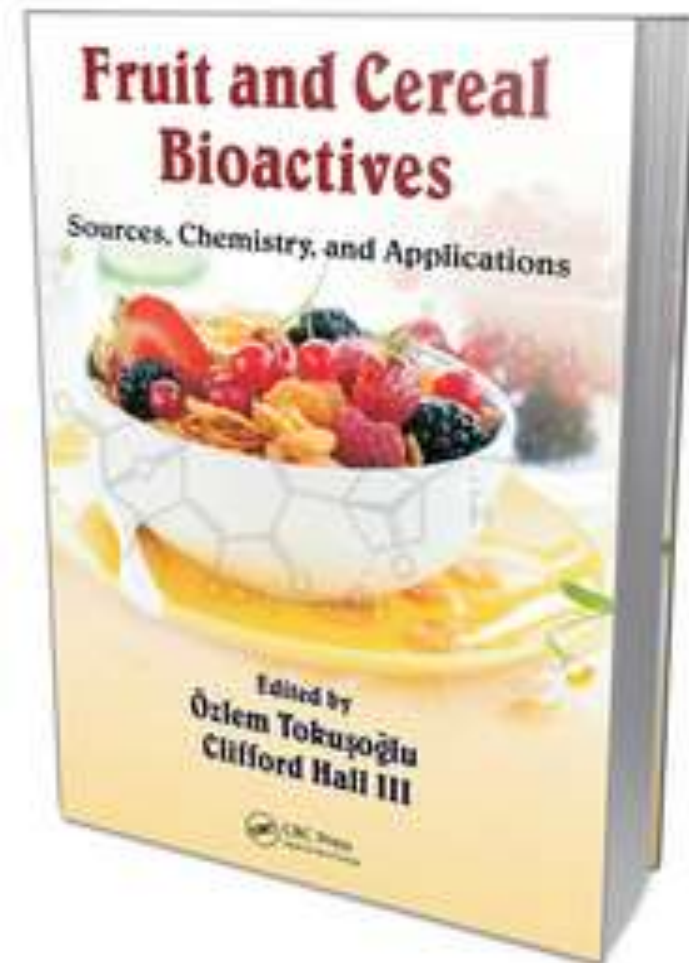
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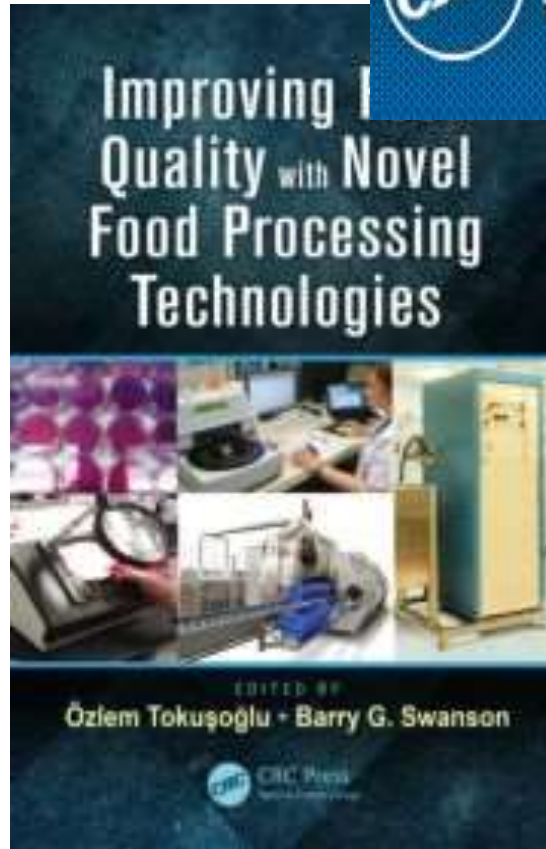
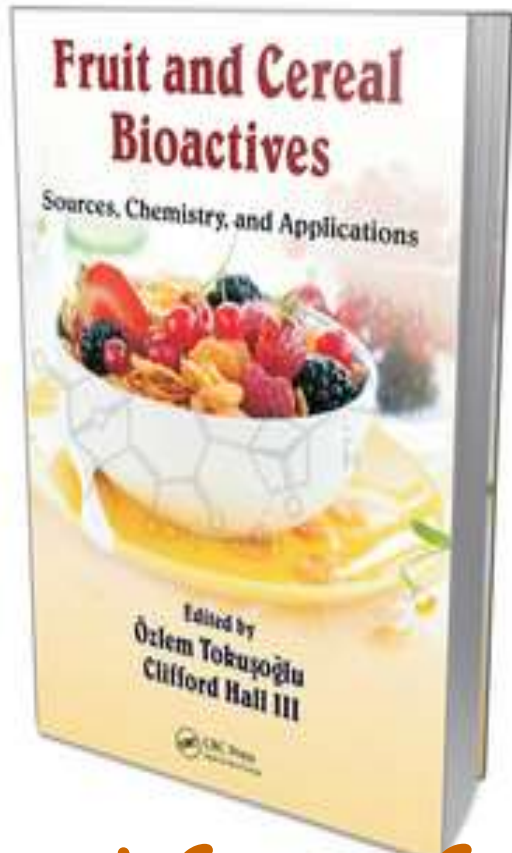
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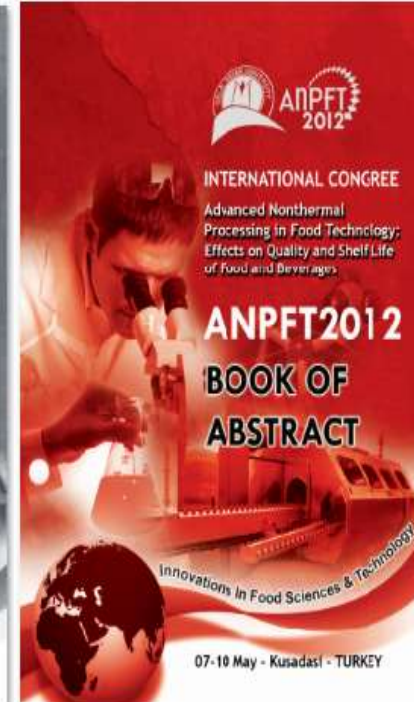
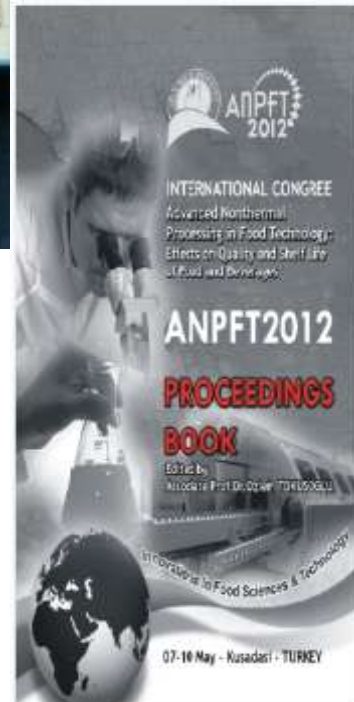


Chapter **23** **High Pressure Technology on Bioactives in Fruits & Cereals**

**Özlem Tokuşoğlu & Christopher J. Doona**



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