Extract from pomegranate waste as an alternative natural antioxidant in foods

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Pomegranate

Pomegranate is an ancient fruit originating from the Middle East and nowadays the global pomegranate production is around 2 million tons

The most important growing regions are:

- China
- Iran
- Egypt
- Turkey
- Spain
- U.S.A.

24% Peel

14% Seeds

62% J

Composition-Polyphenol Content of Pomegranate Peel

Component	Content (%)
Total solids	96.00
Moisture	4.00
Total sugars	31.38
Proteins	8.72
Crude Fiber	21.06
Fat	9.40
Ash	5.00
Total phenolics	8.10

Aguilar et al., 2008; Ullah et al., 2012

- Antioxidant activity
- Anti-mutagenic activity
- Anti-hypertension activity
- Anti-inflammatory activity
- Anti-atherosclerotic activity

Phenolic compound	Content (mg/100 g dry matter)
Ellagic acid	44.19
Catechin	868.40
Punicalagin	1667.00
Gallic acid	125.80
Protocatechol	4.17
Vanilline	3.91
Caffeic acid	60.46
Ferulic acid	5.89
p-coumaric acid	17.64
Others	8.20

Rowayshed et al., 2013; Gullon et al., 2016

Exploitation of Pomegranate Peels

Pomegranate 100 kg

Peels 24 kg

Seeds 14 kg

Fodder

Food Industry

- Ice cream
- Tea

Cosmetics

Proposed Process for Pomegranate Peels Application in Food Industry

Pomegranate Peels

Drying

Solvent

Grinding

Ultrasound-assisted extraction

Fodder or Biosorbent

Filtration

Recycled solvent

Evaporation

Food additives

Wall material

Drying

Phenolics

Emulsification

Microcapsules of phenolics

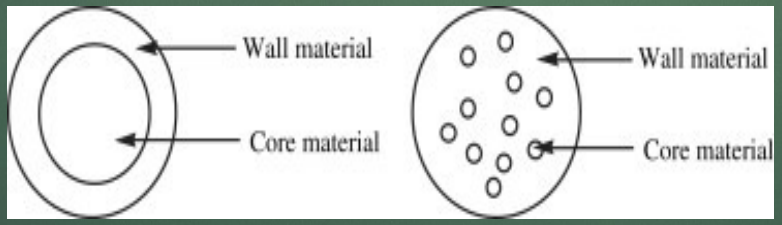
Encapsulation by spray drying

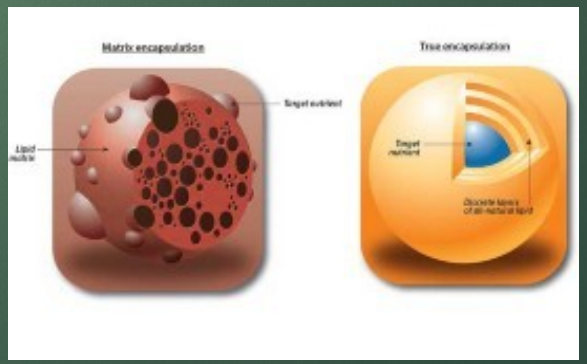
Kaderides et al., 2015

Why Encapsulation of Phenolic Compounds

- Increase of their stability during storage and passage through the gastrointestinal tract
- Improvement of color
- Masking of astringency
- Suitability for use as an additive in functional foods

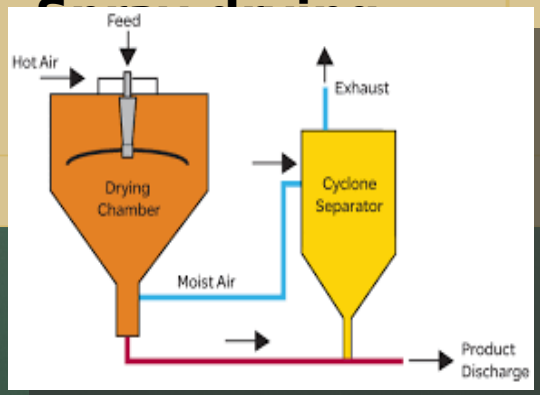
Fang & Bhandari, 2010

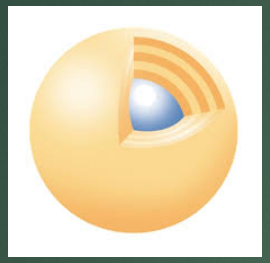




Encapsulation Methods

Method of encapsulation	Encapsulation efficiency	Reference
Extrusion	89.39%	Belščak-Cvitanovic et al., 2011
Rapid extraction of supercritical solution	79.78%	Santos et al., 2013
Formation of multiple emulsion using a rotating disk reactor	80.00%	Akhtar et al., 2014
Freeze drying	75.50% 97.22%	da Rosa et al., 2014 Saikia et al., 2015
Freeze drying liposomes	63.19%	Marin et al., 2018
Spray drying	72.40% 99.80%	Bustamante et al., 2017 Kaderides et al., 2015





Wall material characteristics

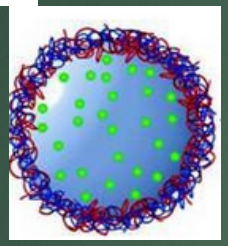
1. Good rheological properties at high concentration
2. Disperse or emulsify the active material and stabilize the emulsion produced
3. Chemical non reactivity with the active core materials
4. Seal and hold the active material within its structure during processing/storage
5. Provide maximum protection to the active material against environmental conditions
6. Acceptable solubility of the solvent to the food industry

Desai & Park, 2005

Wall Materials Used for Encapsulation of Phenolics

Phenolic extract	Wall material	References
Spray drying		
Pomegranate peel extract	Maltodextrin; Whey protein; Skim milk powder Maltodextrin Modified starch	Kaderides et al., 2015 Cam et al., 2014 Bustamante et al., 2017
Carrot extract	Maltodextrin	Ersus &Yurdagel, 2008
Grape juice	Maltodextrin; Soy protein; Whey protein	Moser et al., 2017
Olive leaf extract	Chitosan	Kosaraju et al., 2006
Barberry extract	Maltodextrin; Gum Arabic; Gelatin	Mahdavi et al., 2016
Mutle plant extract	Maltodextrin; Gum Arabic; Mesquite gum	Pavón-García et al., 2011
Soybean extract	Maltodextrin; Starch	Georgetti et al., 2008
Black currant extract	Maltodextrin; Inulin	Bakowska & Kolodziejczyk, 2011
Bilberry extract	Whey protein concentrate	Betz et al., 2012
Apple extract; Olive leaf extract	Sodium caseinate; Lecithin	Kosaraju et al., 2008
Co-crystallization		
Yerba Mate extract	Calcium alginate	Deladino et al., 2008
Green tea EGCG extract	Gelatin	Shutava et al., 2009
Blackcurrant extract	Glucan	Xiong et al., 2006
Freeze drying		
Cloudberry extract	Maltodextrin DE 5-8 & DE18,5	Laine et al., 2008
Grape pomace extract	Maltodextrin; Gum Arabic	Stoll et al., 2016
Blueberries extract	Maltodextrin	Celli et al., 2016
Hibiscus tea extract	Pullulan	Gradinaru et al., 2003

Wall Materials Suitable for Encapsulation of Phenolic Compounds



Carbohydrates

Proteins

Undesired taste

Unnatural additives

Wall material with appropriate encapsulation properties

Replacement with alternative products

Insoluble dietary fibres

Complex carbohydrates

Strong absorption properties

Natural dietary fibres

Soluble dietary fibres

Assist regular bowel movements and flushing the intestinal system of undesirable materials

Insoluble dietary fibres

Slow down the metabolism rates of sugars and form a lining gel within the intestines

Production and Exploitation of Orange Fruit

Production of oranges in E.U.

Country	Tons (2013)
Spain	2.933.800
Italy	1.950.000
Greece	914.000
Portugal	206.300

Highly polluted wastewater

50% by-product (peel, seed, pulp)

50% juice

Wastes

Food industry
(source of dietary fiber)

Animal feed

Fertilizer

No economic value

Incorporation of Pomegranate Peel Extract in Foods

Product	Activity	Reference
Encapsulated pomegranate peel extract		
Hazelnut paste	Antioxidant	Kaderides et al., 2015
Ice cream	Antioxidant	Cam et al., 2014
Pomegranate peel extract		
Shrimps	Antimicrobial	Basiri et al., 2015
Meat pate	Antimicrobial	Hayrapetyan et al., 2012
Curd	Antioxidant	Sandhya et al., 2018
Pork meat	Antioxidant	Qin et al., 2013
Beef meatballs	Antioxidant	Turgut et al., 2017
Bread	Antioxidant	Paari et al., 2012; Altunkaya et al., 2013
Sunflower oil	Antioxidant & Antimicrobial	Iqbal et al., 2008; Kanatt et al., 2010
Cooked chicken patties	Antioxidant & Antimicrobial	Naveena et al., 2008

Incorporation of Pomegranate Peel Extract in

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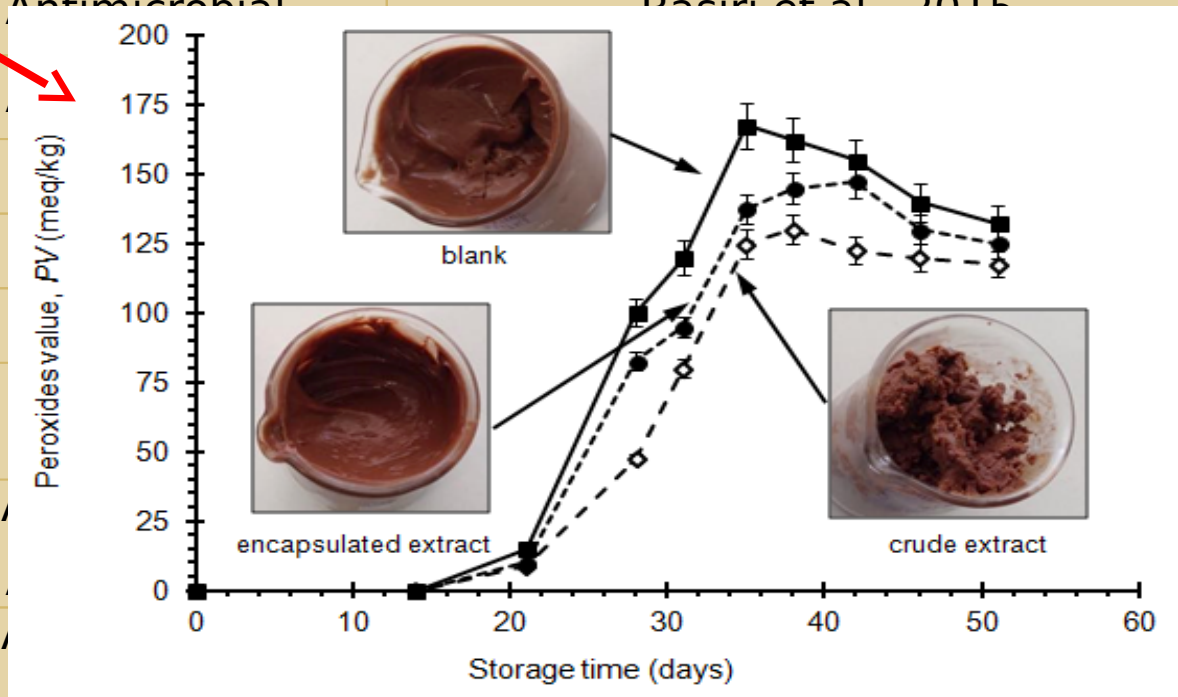
Curd		
Pork meat		
Beef meatballs		
Bread		013
Sunflower oil		L0
Cooked chicken patties	Antimicrobial	



Objective

The exploitation of pomegranate and orange wastes *based on:*

- *Ultrasound-assisted extraction of phenolic compounds from pomegranate peel*
- *Encapsulation of extract by spray drying using orange juice industry by-product as wall material*

Study of:

Incorporation of crude and encapsulated extract in foods:

a. *Fresh juice*
b. *Sunflower oil*
c. *Cookies*

Integrated Process for Orange Wastes Application as Wall Material for Encapsulation

Orange

Juice production → Juice

Orange wastes

Hot water – 100 °C → Boiling — 20 min

Washing

Drying — 60 °C, 48 h

Grinding

Sieving

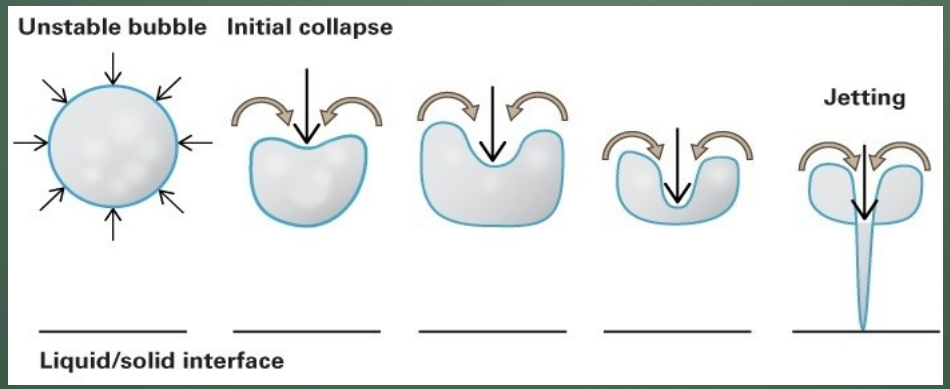
Orange wastes powder

Pomegranate Peel Extract – Optimized Conditions of Ultrasound Assisted Extraction

1. **Extraction temperature:** *35 °C*
2. **Solvent type:** *Water*
3. **Solvent/Solid ratio:** *32/1*
4. **Amplitude level:** *40% (50 W)*
5. **Pulse duration/Pulse interval ratio:** *7/6*
6. **Extraction time:** *10 min*

130 W, 20 kHz VCX-130 Sonics and Materials (Danbury, CT, USA) with Ti–Al–V probe (13 mm)

Kaderides et al., 2015



Integrated Process for Encapsulation of Pomegranate Peel Extract in Orange Wastes Powder

Encapsulation of Pomegranate Peels Extract – Optimized Conditions of Spray Drying

1. **Wall material:** Orange wastes
2. **Inlet air temperature:** 162 *°C*
3. **Feed solids concentration:** 5 % w/w
4. **Ratio of core to wall material:** 1/9
5. **Drying air flow rate:** 17.5 m^3/h
6. **Flow rate of compressed air for atomization:** 0.80 m^3/h

- Concurrent
- Two – fluid nozzle atomization
- Peristaltic pump for feed

Buchi, B-191,
Buchi Laboratoriums-Technik,
Flawil, Switzerland

Incorporation of Phenolic Capsules in Foods

- **PRODUCT:**

a. Fresh juice → Shelf-life test at 4°C for 20 days

b. Sunflower oil → Shelf-life test at 60°C for 20 days

c. Cookies → Shelf-life test at 25°C for 21 days

- **ADDITIVE:** Phenolic extract (Crude and encapsulated)

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3 samples were tested:

- Sample with encapsulated extract
- Sample with crude extract
- Control sample

Measurement of:

- ✓ Antioxidant activity
- ✓ Total phenolics conten
- ✓ Oxidation stability
- ✓ Color

Foods Composition Data

Shelf-life test at 25°C for 21 days

Component	Cookies with crude extract	Cookies with encapsulated extract
Phenolics concentration: 5000 ppm		
Flour	42.82 %	44.68 %
Butter	29.17 %	29.67 %
Sugar	14.12 %	14.73 %
Baking powder	1.39 %	1.36 %
Crude extract	12.75 %	-
Encapsulated extract	-	9.55 %

Shelf-life test at 4°C for 20 days

Fresh juice
Phenolics concentration: 5000 ppm
Apple
Orange
Carrot

Sunflower oil

- Phenolics concentration: 500 ppm
- Shelf-life test at 60°C for 20 days
- Phenolics concentration: 1000 ppm

Results

Extraction and Encapsulation Yield by Ultrasound-Assisted and Spray Drying Method

Optimum conditions for maximum extraction yield

Solvent	T (°C)	A (%)	t (min)	Pulse (sec/sec)	L/P
Water	35	40	10	7/6	32/1

Yield: 13.85 mg GAE/g DM

Optimum conditions for maximum encapsulation yield and efficiency

Wall material	T (°C)	Q_a (%)	Q_s (m^3/h)	w/c
Orange wastes powder	162	50	0.80	9/1

Yield: 12.99%

Efficiency: 99.77%

Microencapsulation yield (Y)

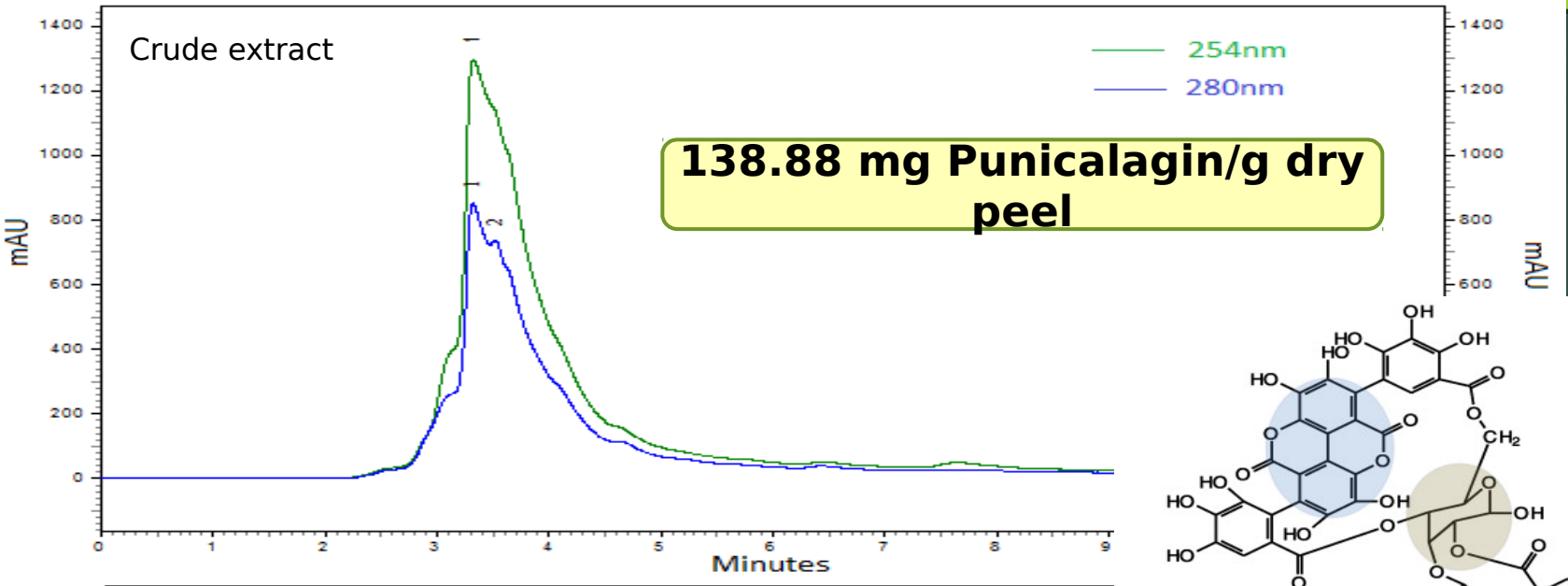
$$Y(\%) = \frac{\text{Mass of microcapsules (g)}}{\text{Total mass of initial substances (g)}} * 100$$

Solids feed collected in product container

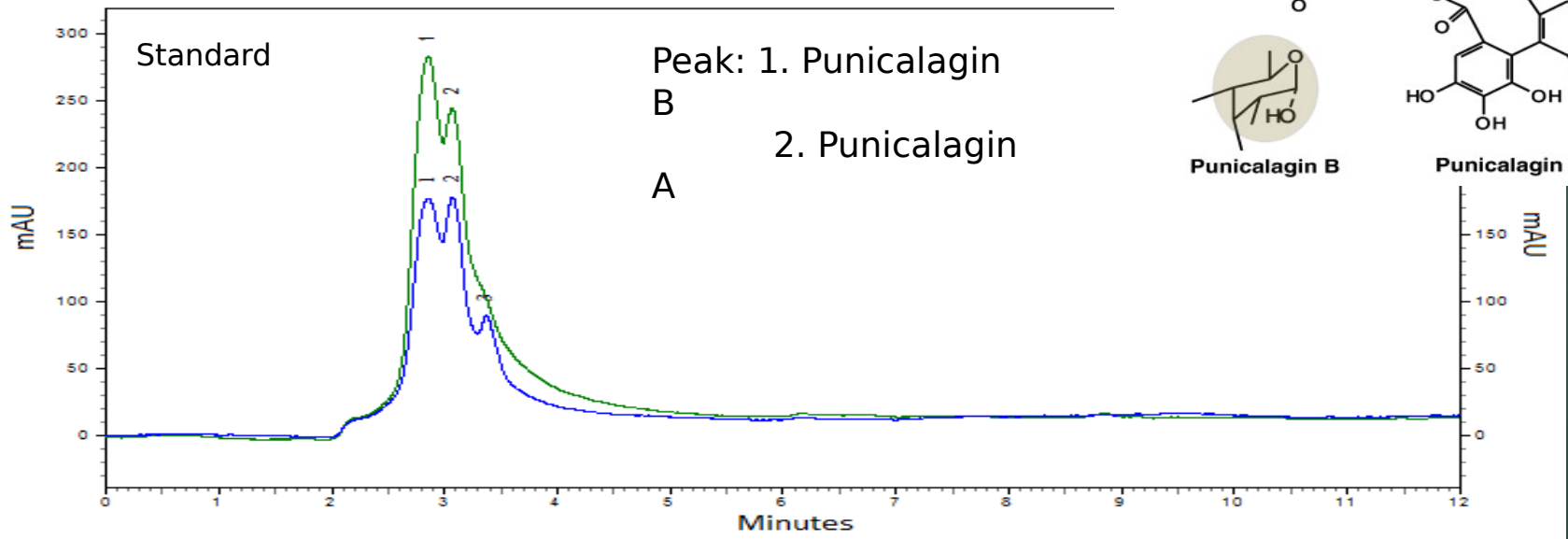
Microencapsulation efficiency (E)

$$E(\%) = \left(1 - \frac{\text{Phenolics on microcapsule surface}}{\text{Total phenolics of microcapsule}}\right) * 100$$

HPLC Analysis of Crude Extract



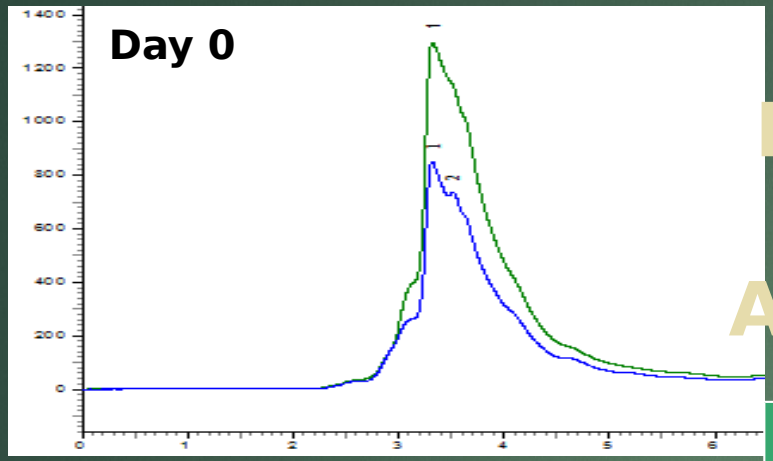
138.88 mg Punicalagin/g dry peel

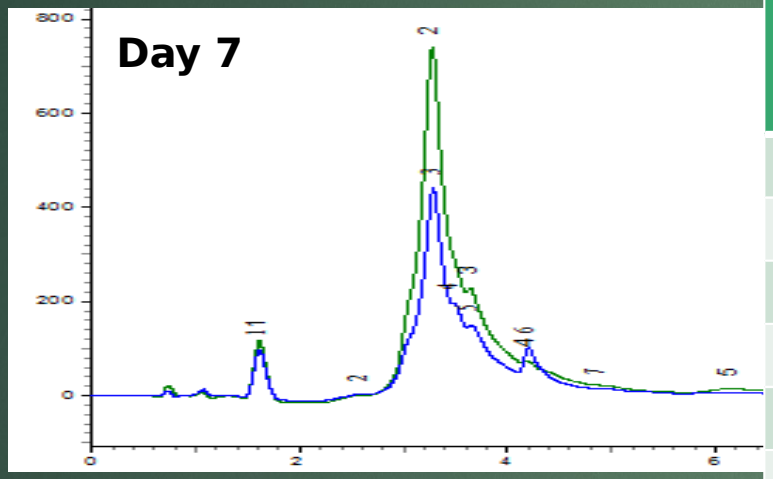


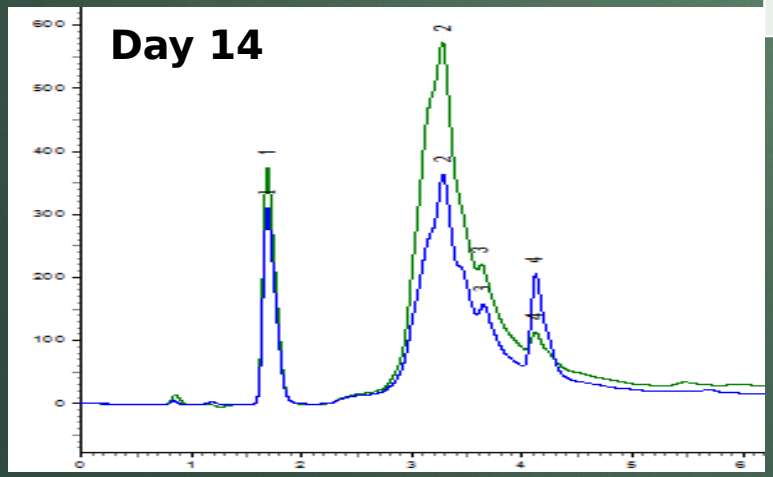
Peak: 1. Punicalagin B

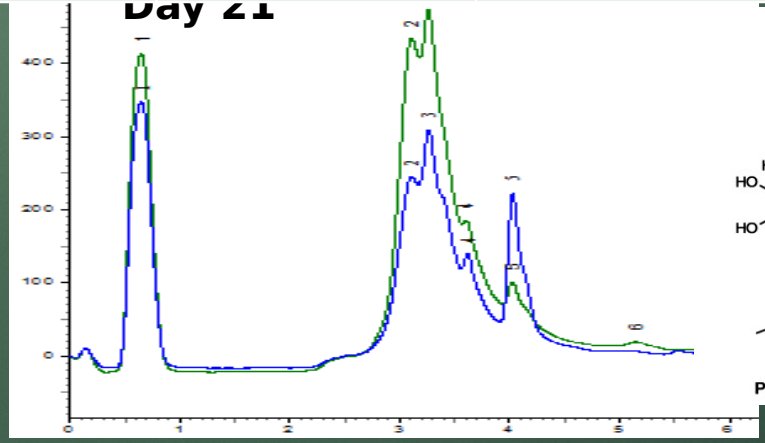
2. Punicalagin A

Determination of Punicalagin in Extract During Storage at Accelerated Conditions

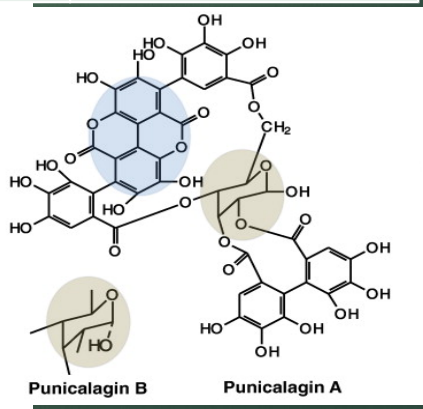








Time (day)	Punicalagin (mg/g dry peel)	Scavenging capacity (%)	Total Phenolics (mg GAE/g dry peel)
0	138.88	94.78	119.82
7	77.12	91.92	83.97
14	85.15	85.38	79.87
21	75.83	84.93	72.82
30	74.54	88.73	85.48
40	70.45	82.57	91.84



Determination of Punicalagin in Extract During Storage at Accelerated Conditions

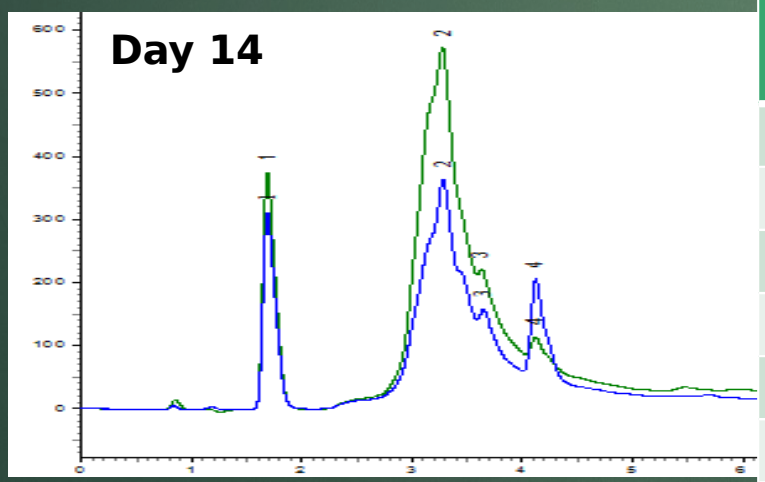
Antioxidant Activity of Isolated Phenolics Measured by DPPH Method[a]

compd	antioxidant activity TEAC[a]	AEAC[b]
gallic acid	2.5	2.7
cyanidin 3 glucoside	0.8	0.8
ellagic acid	1.1	1.2
punicalagin	6.3	6.7

Antioxidant Activity of Individual Phenolic Groups in Commercial Single-Strength Pomegranate Juice Measured by the DPPH Method

phenolic groups	antioxidant activity TEAC	AEAC
anthocyanins	1.4	1.4
punicalagins	9.8	10.5
ellagic acids	0.5	0.6
hydrolyzable tannin	6.2	6.6

Gil et al., 2000



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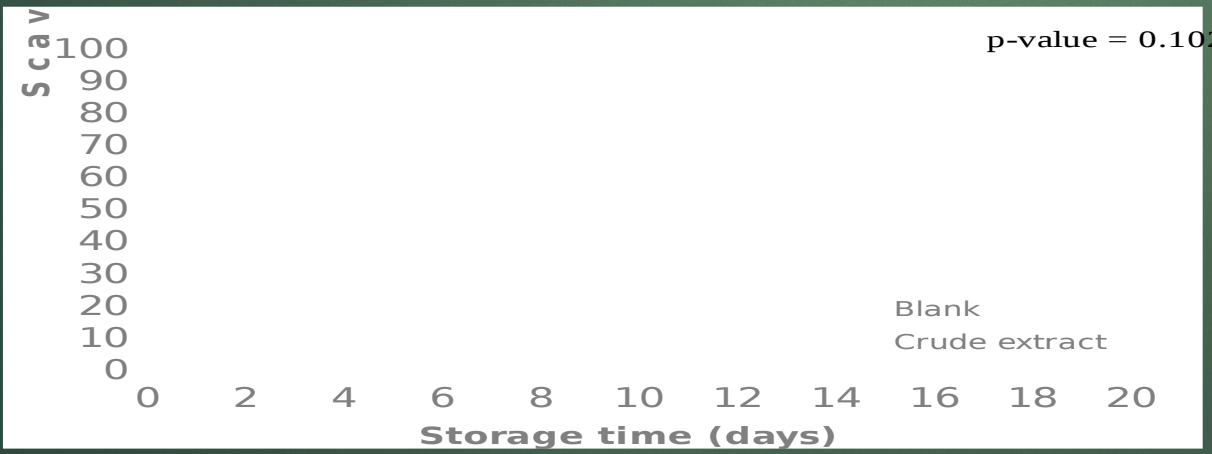
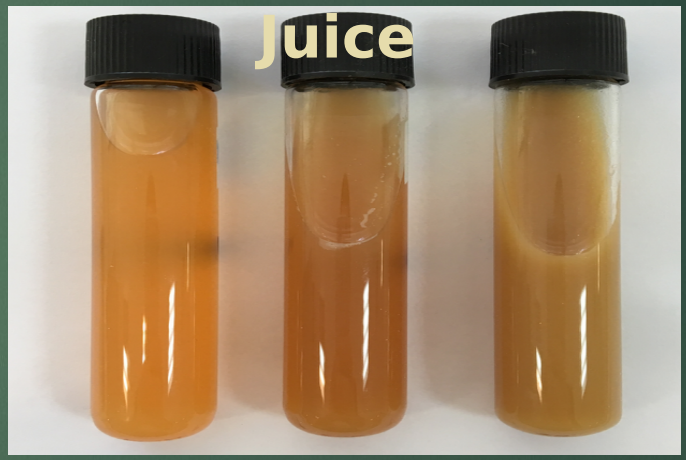
40	70.45	82.57	91.84

Effect of Pomegranate Peel Extract on Total Phenolics and Antioxidant Capacity of Fresh Juice



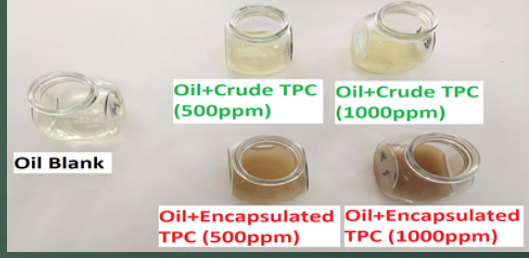


Blank | Crude extract | Encapsulated extract

Shelf-life test at 4°C for 20 days

Antioxidant Capacity- Peroxide Value of Sunflower Oil

Shelf-life test at 60°C for 20 days



Peroxide Value (meq O2/kg

P-value=0.000

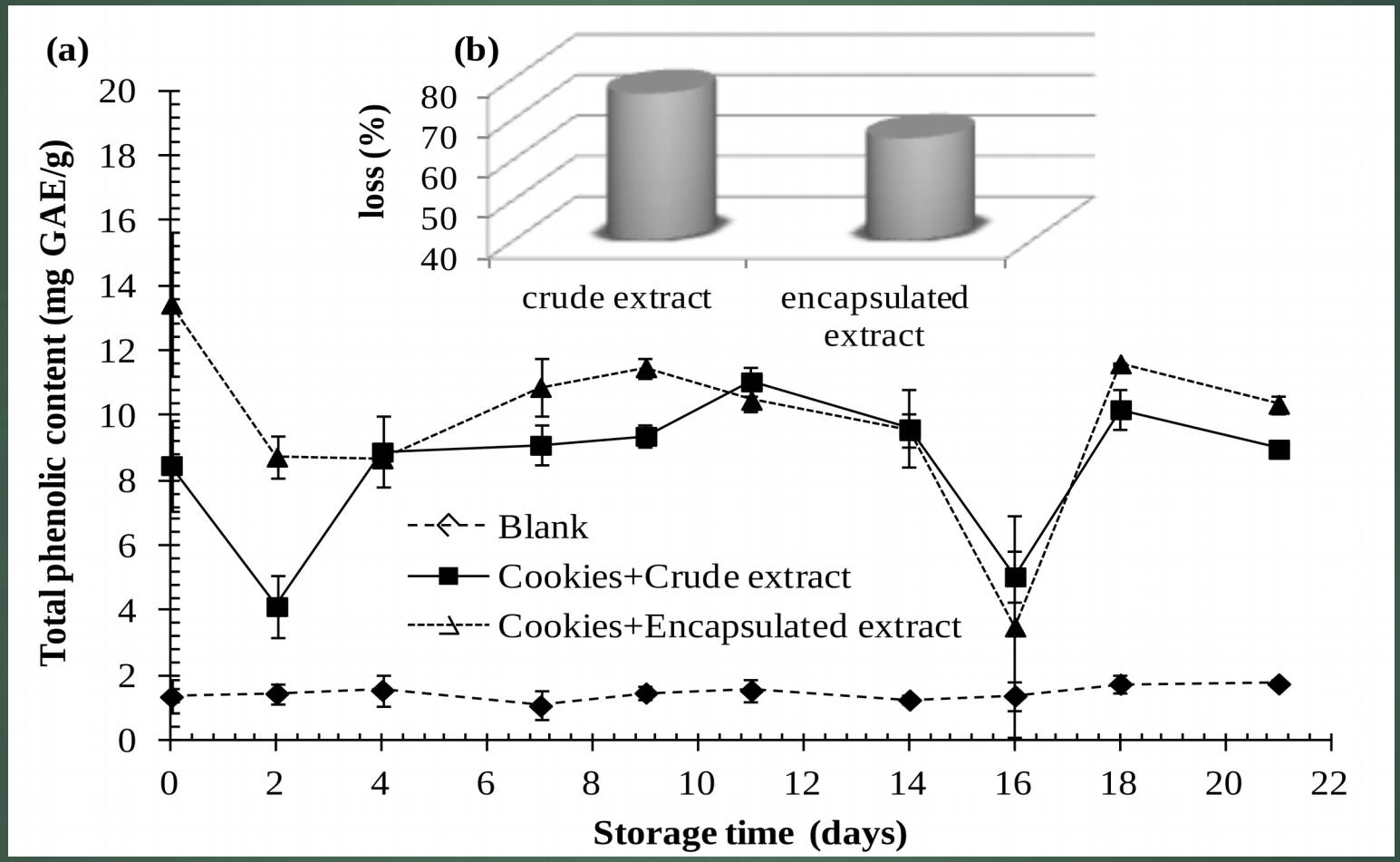
Peroxide Value (meq O2/kg

p-value=0.000

p-value=0.000

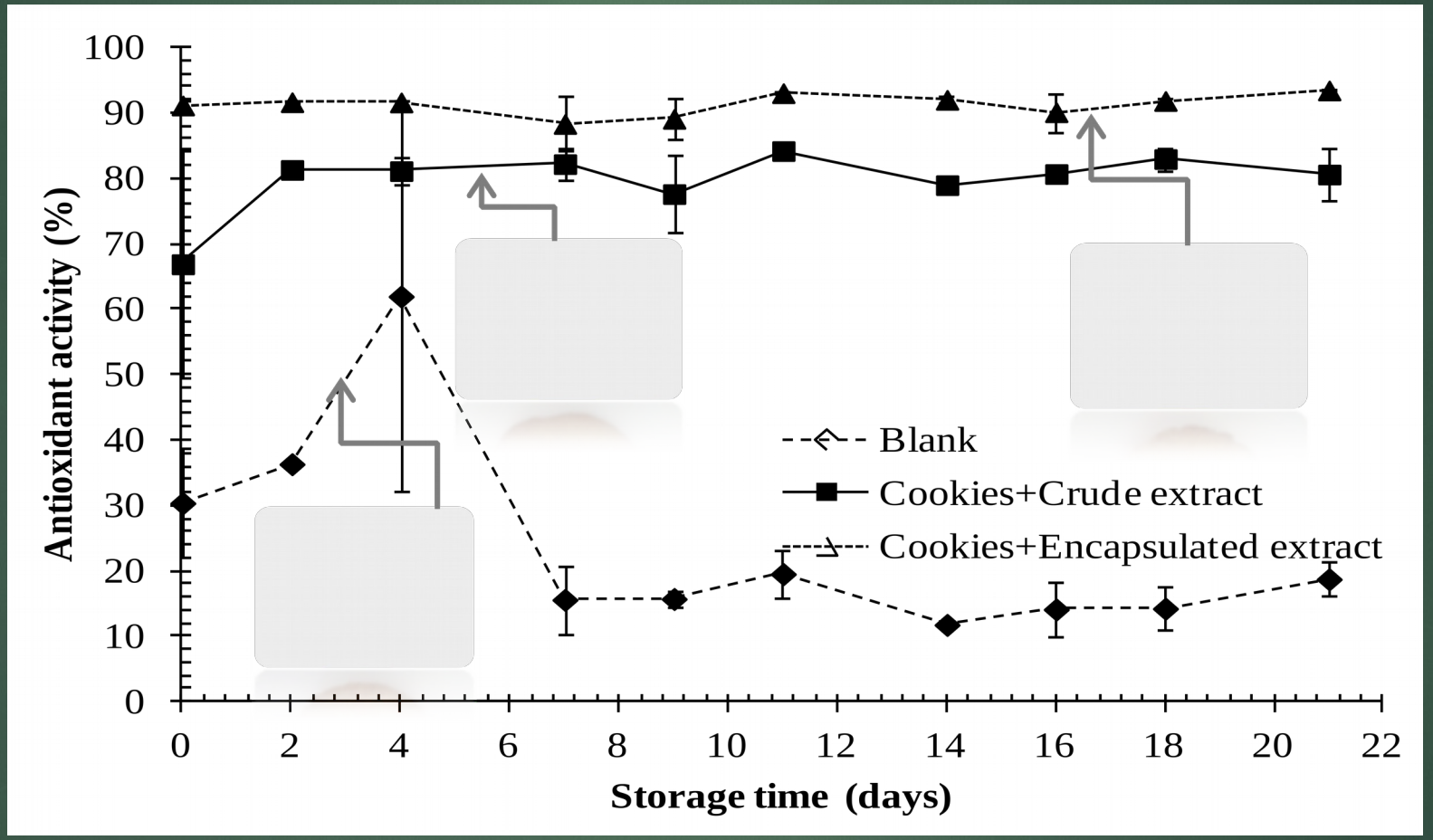
p-value=0.000

Effect of Pomegranate Peel Extract on Total Phenolic content of Cookies



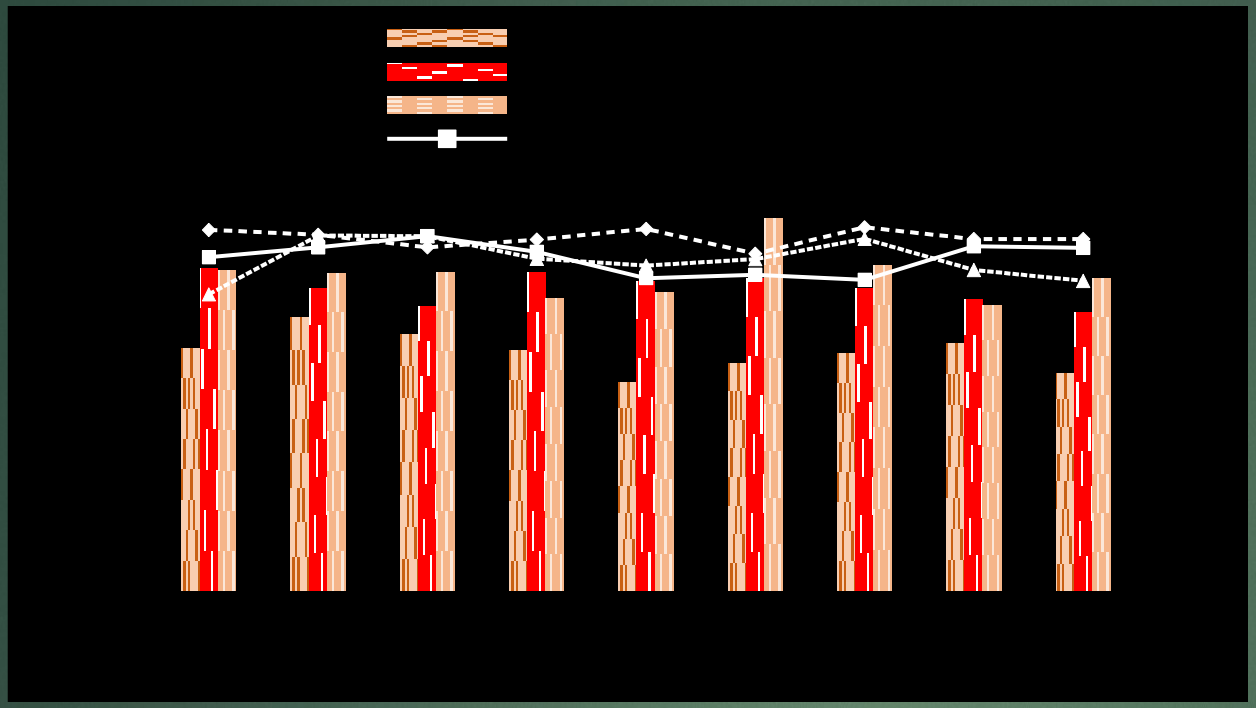
Shelf-life test at 25°C for 21 days

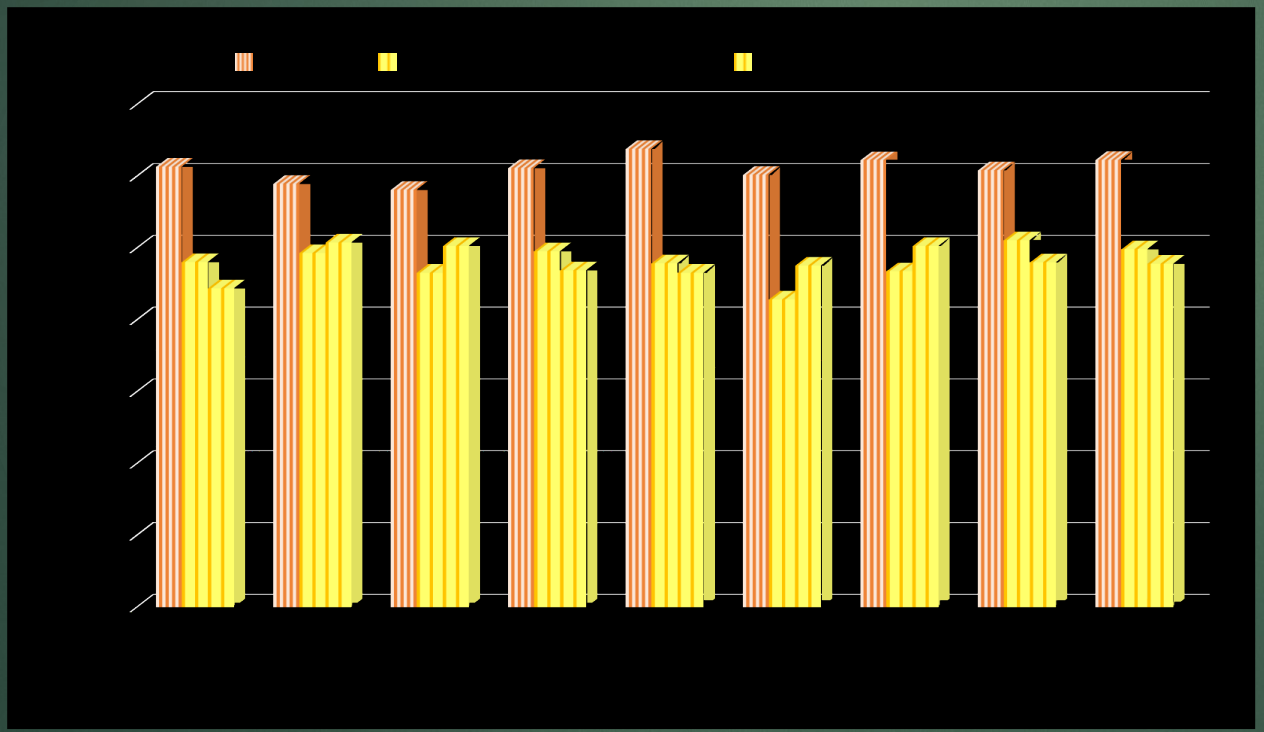
Effect of Pomegranate Peel Extract on Antioxidant Capacity of Cookies



Shelf-life test at 25°C for 21 days

Effect of Pomegranate Peel Extract on Color of Cookies





L*: Lightness

a*: (+) red, (-) green

b*: (+) yellow, (-) blue

Shelf-life test at 25°C for 21 days

Effect of Pomegranate Peel Extract on Sensory Evaluation of Cookies

Triangle test

$$z - score = \frac{(P_{obs} - p) - 1/2n}{\sqrt{pq/n}} = \frac{(X - np) - 1/2}{\sqrt{npq}}$$

where Pobs is the proportion correct (= X/n), X is the actual number correct, n is the number of judges, p is the chance probability, and q = 1−p.

Solving for X as a function of n and using p = 1/9 and z = 1.645 ($p < 0.05$, one tailed)
For **n = 10** panelists, **X = 3.246**.
The value of X is round up to the next highest integer. Therefore, in the experiments conducted, it will be assumed that the samples have statistically significant difference, if **4** or more panelists responded correctly in both iterations.

Component	Cookies with crude extract	Cookies with encapsulated extract
Phenolics concentration: 5000 ppm		
Flour	42.82 %	44.68 %
Butter	29.17 %	29.67 %
Sugar	14.12 %	14.73 %
Baking powder	1.39 %	1.36 %
Crude extract	12.75 %	-
Encapsulate	-	9.55 %

Astringency

	1st Test	2nd Test
Cookies with crude extract	0	0
Cookies with encapsulated extract	10	10

Bitterness

	1st Test	2nd Test
Cookies with crude extract	1	0
Cookies with encapsulated extract	9	10

Overall acceptability

	1st Test	2nd Test
Cookies with crude extract	10	10
Cookies with encapsulated extract	0	0

Effect of Pomegranate Peel Extract on Sensory Evaluation of Cookies

Συστατικό	Cookies with crude extract	Cookies with encapsulated extract	Cookies with encapsulated extract
Συγκέντρωση φαινολικών 5000 ppm			
Flour	42.82 %	44.68 %	34.11 %
Butter	29.17 %	29.67 %	22.66 %
Sugar	14.12 %	14.73 %	18.75 %
Baking powder	1.39 %	1.36 %	1.04 %
Crude extract	12.75 %	-	-
Encapsulated extract	-	9.55 %	9.38 %
Water	-	-	14.06 %

Color	
Cookies with crude extract	3
Cookies with encapsulated extract	9

Texture	
Cookies with crude extract	9
Cookies with encapsulated extract	3

Overall acceptability	
Cookies with crude extract	7
Cookies with encapsulated extract	5

Odor	
Cookies with crude extract	1
Cookies with encapsulated extract	11

Taste	
Cookies with crude extract	7
Cookies with encapsulated extract	5

3

2

Conclusions

An integrated approach for utilization of pomegranate peels is suggested based on the spray drying encapsulation of their phenolics compounds using an alternative wall material (orange wastes)

- Pomegranate peel extract was found to be rich in punicalagin
- The extract (crude and encapsulated) was found efficient in improving the shelf-life of sunflower oil, cookies and fresh juice
- On sensory evaluation, about 70-90% of the panelists preferred cookies with encapsulated extract for their **color** and **odor** compared to cookies with uncoated extract, whereas 58% of the panelists preferred cookies with crude extract for **taste** and **overall acceptability**

Phenolics

Fibers

Thank you!

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