



**Antimicrobial, antioxidative,
phytochemical and functional
characteristics of the extracts of some
plants seeking to adapt them as a potential
source of biologically active substances for
the safety of dairy products**

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Research topics at the Department of Food Quality and Safety



Veterinary Academy, Department of Food Safety and Quality

Research topics

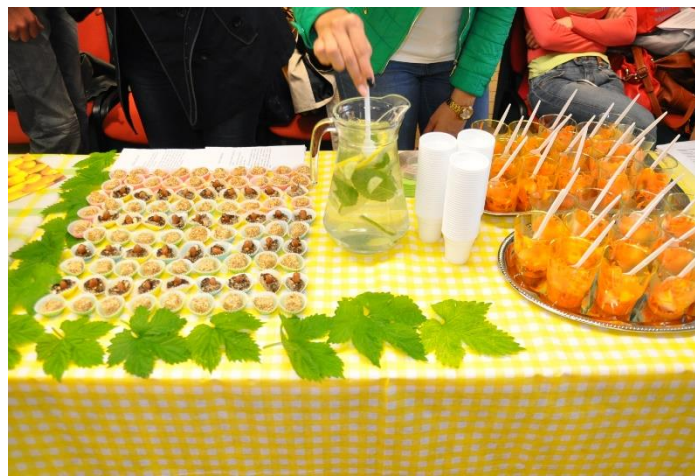
- Microbiological food safety
- Food microbiology
- Innovations in food technologies
- Mycotoxins in feed and food
- Quality and safety of plant, meat and dairy products (whey and buttermilk products)
- Functional food (with herbs extracts)
- Animal health and welfare



Events organised by Department of Food Safety and Quality

- Since 2013: festival „Healthy and Safe Food“
 - Organised by FSQ and students
 - Veterinary Food Safety, Veterinary Medicine, Food Science
 - For public, students and university staff







Functional food

- Food consumers expect from food products good gustatory characteristics, stability during the period of consumption and storage, and compliance with healthy nutrition requirements.



Functional food

- Functional food are rising food markets around the world and are expected to belong to the emerging trends for food industry in the new millennium.
- The research opportunities in nutrition to explore the relationship between a food or a food component and an improved state of health and well-being, or reduction of disease, present the greatest challenge to scientists now and in the future.



Functional food

- Many chronic diseases are diet related and more than 25-70% of the diseases may be prevented by optimal food intake, due to specific and balanced food ingredients.
- For this purpose consumer should be able to make food choices based on clear and accurate information.



Dairy Food and Herbs

- One of the most important research areas in the dairy industry regarding the development of dairy products and their scientific research is the enrichment of the products with various active components (Ballard and Morrow, in 2013; Lonnerdal, 2004; Martin et al., 2012). This is related to the fact that consumers tend to avoid nutrition related mistakes since public awareness as to the connection of nutrition and health is increasing (Sharma et al., 2011). Extracts of various plants and spices is the area of focus of the above-mentioned active components (Chandrani et al., 2012). This is a renewable source of biologically active substances characterised by aromatic and anti-oxidizing and anti-microbial properties (Dai and Mumper, 2010).

Antimicrobial, antioxidative, phytochemical and functional characteristics of the extracts of some plants seeking to adapt them as a potential source of biologically active substances for the safety of dairy products



Aim of the study:

to evaluate antimicrobial, antioxidative, phytochemical and functional characteristics of the extracts of some plants seeking to adapt them as a potential source of biologically active substances for the safety of dairy products.



We used chinese hibiscus (*Hibiscus rosa-sinensis L.*) and honeybush (*Cyclopia intermedia*) plant for preparation of extracts with different methodology and polarity of solvents and evaluate their antimicrobial and antioxidant activity in model systems.



Healing Properties of Chinese Hibiscus

- Chinese Hibiscus has antimicrobial, hypoglycemic, astringent, anti-inflammatory, refrigerant and aphrodisiac properties. It has a flavonoid known as cyanidin, which has antioxidant as well as anti-inflammatory qualities.





Chinese hibiscus for food industry

- Antioxidant activity, antibacterial properties of flowers were investigated(Yin Wei Mak, 2012).
- Leaves and petals of *H. rosa sinensis* has the strongest antioxidant activity. Leaves and petals of *H.rosa-sinensis* developed into functional food and skin care products.(Divyja M.J. et al, 2013).



Chinese hibiscus for food industry

- Natural anthocyanins from *Hibiscus rosa sinensis* flowers widely used as coloring agents for many food products. Extracts are used for taste acid flavor, color, many healing compounds.

Honeybush has amazing healing properties





Honeybush tea

- Honeybush research is exploring the healing properties, the richness of bioactive compounds and especially the pleasant taste.





Honeybush tea

- Plants are traditionally used for the production of the South African herbal tea, honeybush, and recently as aqueous extracts for the food industry.
- *Cyclopia intermedia* aqueous extract and mangiferin (a major constituent) are known to have anti-diabetic properties. Variation in phenolic composition and antioxidant capacity is expected due to cultivation largely from seedlings, having implications for extract standardization and quality control.



Honeybush

- Honeybush infusions are gaining popularity due to their characteristic honey-like flavour, low tannin content, absence of caffeine and potential health effects related to their antimutagenic and antioxidant properties.



Benefits of Honeybush

- 1. High level of antioxidants
- 2. Boost Metabolic System
- 3. Prevents Cancer
- 4. Relieve Asthma
- 5. Treats Cough & Cold
- 6. Prevent Stomach Woes



Benefits of Honeybush

- 7. Heals Sore Throat
- 8. Prevents Bacterial Infections
- 9. Strengthens Liver
- 10. Better Sleep
- 11. Helps In Treating Osteoporosis
- 12. Helps for Treating Depression



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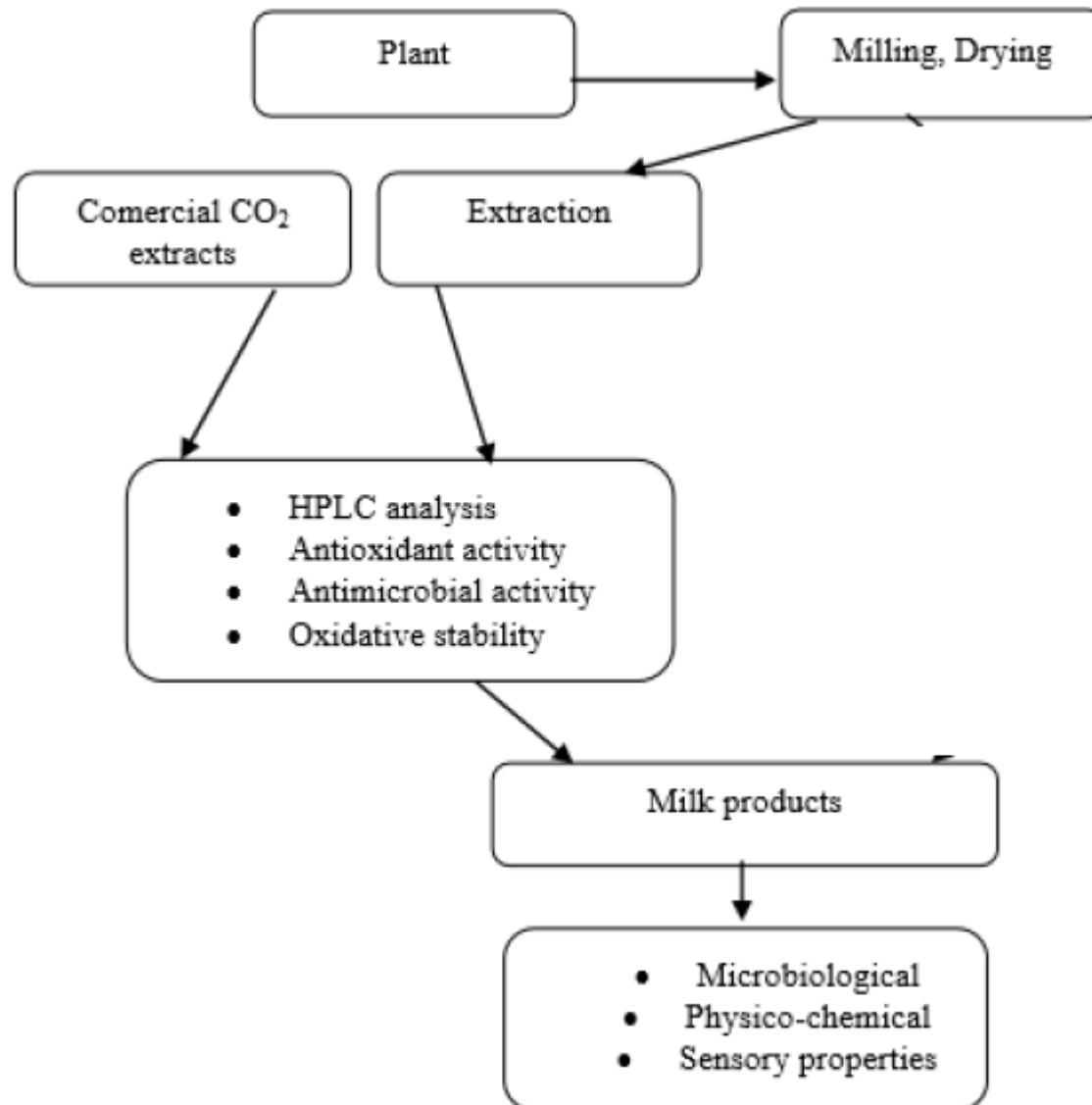
Honeybush in food industry

Honeybush tea



- The extract is used to improve the taste and quality of the confectionery and in beverage industry.

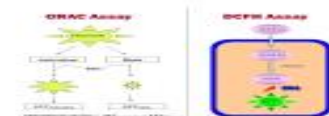
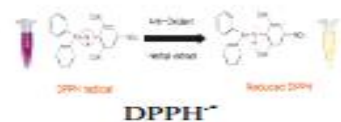
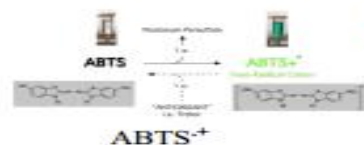
The design of the study



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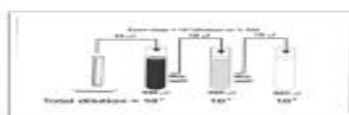
ANTIOXIDANT ACTIVITY



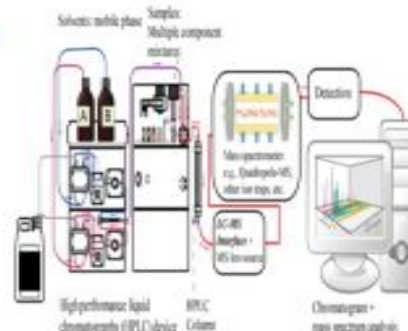
ORAC



ANTIMICROBIAL ACTIVITY



PHYTOCHEMICAL COMPOSITION



UPLC/MS/MS

Curd with honeybush and chinese hibiscus



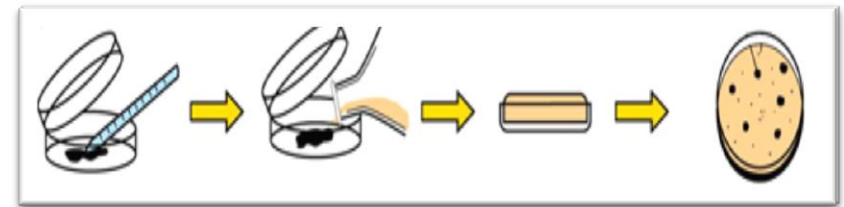
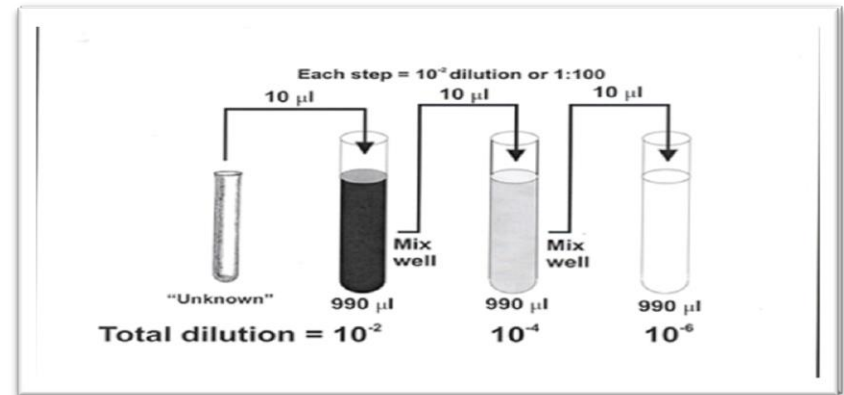
Measurements of antioxidant and radical scavenging activity



- ABTS^{•+} radical cation decolorization assay
- DPPH[•] radical scavenging assay
- ORAC oxygen radical absorbance capacity
- Assessment of Antioxidant Capacity by QUENCHER assay
- Measurement of Total Phenols Content (TPC)

Antimicrobial susceptibility by disk diffusion method:

- Preparation of bacteria inoculums – 18 h at 30-37 °C.
- Inoculums suspension by McFarland No 0.5 Standard ($\sim 1 \times 10^8$ CFU/ml)
- Inoculate Plate Count Agar
- Disc diffusion agar and incubate 24 h at 37° C.
- Classical microbiological methods (according LST ISO EN):
 - *Escherichia coli*
 - *Listeria monocytogenes*
 - *Staphylococcus aureus* count.



Bacteria growth assessment in curd



The suspensions of 0.1 ml overnight cultures in 10 g of sterile curd were prepared. Were added 1% concentration of extracts. Inoculated suspensions without extracts served as control. After inoculation (0 h) and after 5 and 7 day incubation at 30°C and 37°C, serial dilutions were plated on M17 agar and selective media. The plates with starter cultures were incubated at 30 °C for 72 h and 48 h, respectively. The plates with pathogenic bacteria (*E. coli*, *S. aureus* and *L. monocytogenes*) were incubated at 30 and 37 °C for 24h.

Results and discussion





Antimicrobial properties of plant extracts

- The agar well diffusion tests have shown that all extracts has antibacterial properties. It has been noted that the Chinese hibiscus extract had stronger effect on three sample cultures – *Esherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*.
- Honeybush extracts characterized by lower antibacterial properties.



Table 1. Minimum inhibitory concentration of Chinese hibiscus extracts against *E. coli*, *L. monocytogenes* and *S. aureus*.

Organism	Conc. (%)	Acetone extract	Ethanol extract	Water extract
Gram-negative				
<i>E. coli</i>	1	12	13	-
	5	17	19	10
	10	20	22	12
	20	25	25	16
Gram-positive				
<i>S. aureus</i>	1	10	12	-
	5	12	17	-
	10	20	19	13
	20	25	24	15
<i>L. monocytogenes</i>	1	11	9	-
	5	21	12	9
	10	25	19	11
	20	32	22	15



Figure 1. *RSC of Chinese hibiscus and Honeybush extracts measured in the DPPH•, ABTS•+ and ORAC*

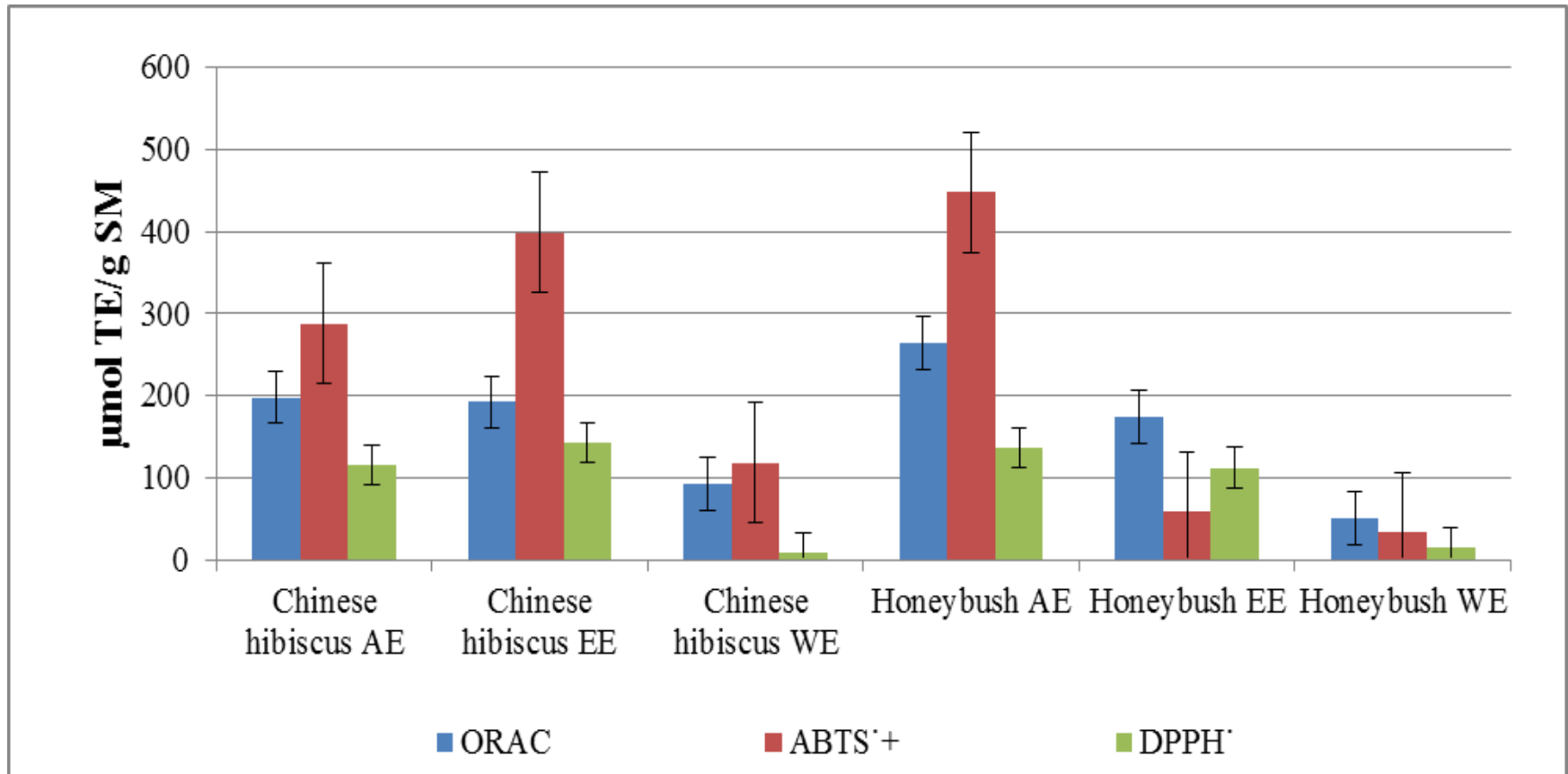
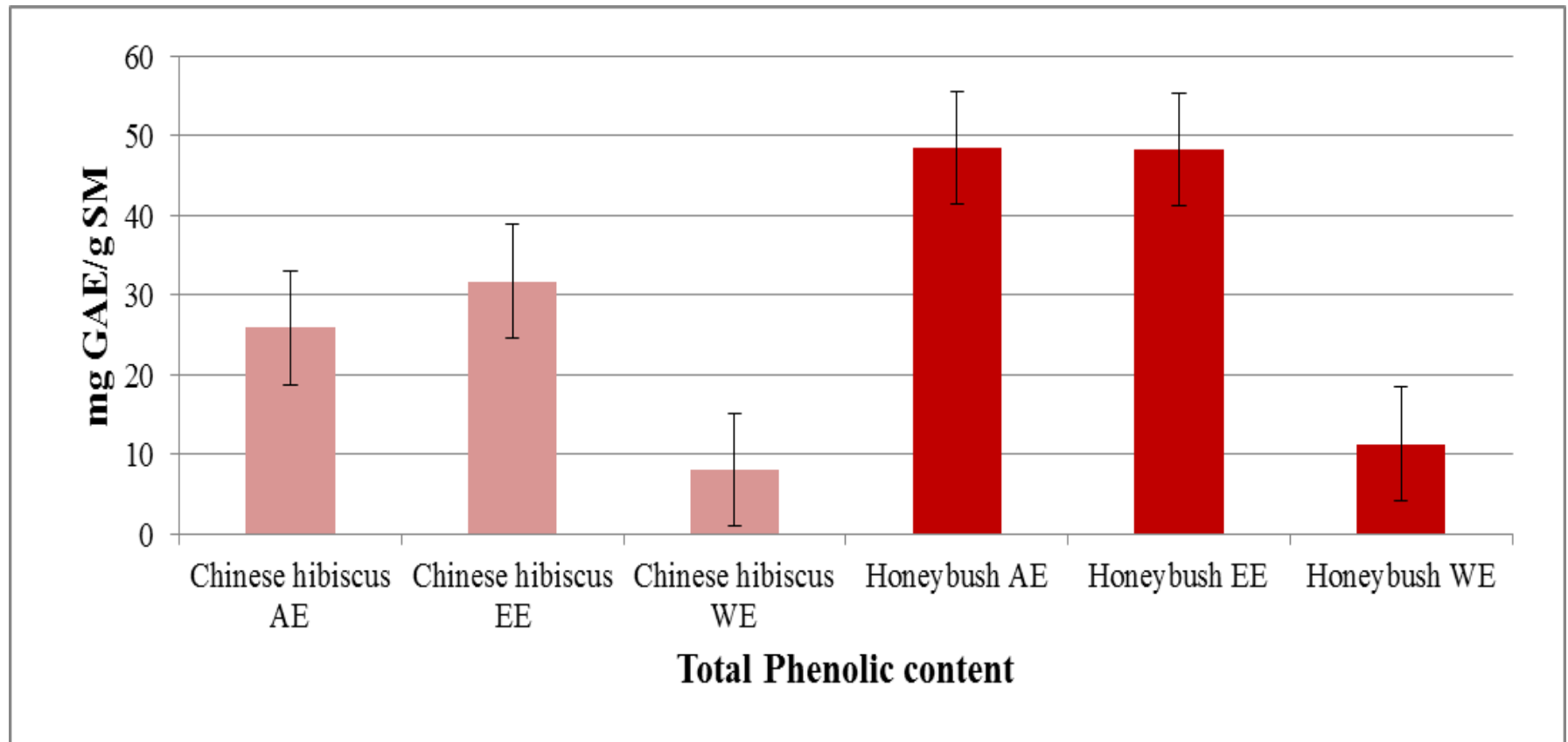




Figure 2. Total amount of phenolic compounds of chinese hibiscus and honeybush extracts



Characterization of honeybush and chinese hibiscus phytochemicals by UPLC/MS analysis



Table 2. UPLC/MS/MS analysis of honeybush and chinese hibiscus extracts.

Tentative identification	Formula (M)	Retention time (min)	MS (M-H), g/mol
Chinese hibiscus extracts			
Quercetin	$C_{15}H_{10}O_7$	4,09	302,24
Campesterol	$C_{28}H_{48}O$	7,84	414,72
Ergosterol	$C_{28}H_{44}O$	7,63	396,65
Pelargonidin	$C_{15}H_{11}O_5$	3,19	271,24
Cyanidin	$C_{15}H_{11}O_6$	6,66	287,24
Honeybush extracts			
Hesperitin	$C_{16}H_{14}O_6$	2,71	302,28
Hesperidin	$C_{28}H_{34}O_{15}$	3,55	610,56
Magniferin	$C_{19}H_{18}O_{11}$	4,61	422,34



Conslusions

1. Sensory indices of fresh cheese with plant additives were acceptable for consumption. Addition of honeybush had the greatest impact on flavor properties of the curd because of well expressed pleasant taste, odor, appearance and antioxidant properties, which increased functionality of the product.
2. Chinese hibiscus extracts could be used in curd production as natural antimicrobial additives against *L. monocytogenes*, *S. aureus* and *E. coli*.



Recomendation

1. We recommend to enrich cheese curd and its products with various extracts, thus creating new and enriching other curd dessert products as well as other confectionery products with curd. Anthocyanins contained in Chinese hibiscus are not only a food dye, they add a product a pleasant color, while other components provide a pleasant sour taste, thus improving the products with antioxidation and antimicrobial activity. The honey bush is a potential antioxidant because of its sweet honey taste and functional properties of its constituents and luteolin.

Science, health and pleasure together





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THANK YOU
FOR
YOUR
ATTENTION
ANY QUESTIONS?