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Original Research Article

Phenolic acids in different preparations of Maize (*Zea mays*) and their role in human health

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A B S T R A C T

The nutritional and therapeutic properties for human health can be attributed to a number of compounds including phenolics. Phenolics are found mostly in plants. Humans and animals lack the pathway responsible for synthesizing phenolic acids which play a great role in human health in several ways. Maize (*Zea mays*) is consumed by people globally and is considered as a good nutrient. However, the analysis of phenolic acids in different preparations of maize (*Zea mays*) has not been done so far. Phenolic acids are synthesized through phenyl propanoid pathway in plant leaves from where they are distributed to entire plant systems. The HPLC analysis of different preparations of maize yielded eight phenolic acids, viz., tannic, gallic, O-coumaric, caffeic, vanillic, ferulic, cinnamic acids and salicylic acid in varying amounts. Maximum number was detected in dry maize grain. Cinnamic and salicylic acids were present in almost all the preparations in sufficient amount along with salicylic acid (13.04μg/g fresh wt.) in corn flour. Gallic acid was present in aqueous extracts of all the preparations except pop corn, stylar portion and baby cob. Out of eight preparations vanillic acid was present only in dry grain, corn flour and maximum in baby cob (121.40 μg/g fresh wt.). Ferulic acid was present in all preparations except pop corn, corn flakes and baby cob. O-coumaric acid was also present in all preparations except baby cob. Several properties, viz., antifungal, antibacterial, allelopathic, immuno-stimulating, antioxidant, inducing resistance in plants etc. have already been reported for several phenolic acids and the same has been connected with the phenolic acids present in different preparations of maize in detail in the present paper particularly with human health.

Keywords

HPLC; phenolic acids; maize; *Zea mays;* O-coumaric; caffeic; vanillic; ferulic; cinnamic acids.

Introduction

Maize (*Zea mays*) is currently produced in several countries of the world and is the third most planted field crops (after wheat and rice). United States, Peoples Republic of China and Brazil are the major producers of maize which together account for 73% of the annual global production of 456.2 million tons. Mexico, (fourth largest producer) currently produces approximately 14 million tons of maize annually on 6.5 million hectares. Phenolics are widely distributed in plants of various categories such as grains, vegetables, fruits and beverages (Laximon-Ramma *et al*., 2005). They exhibit a wide range of biological activities, *viz*., antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, antithrombic, antiviral, anticarcinogenic and vasodialatory actions (Middleton *et al*., 2000; Nardini and Ghiselli, 2003). As phenolics play a great role in human health, they form an integral part of human diet. In the present study phenolic acids were analysed through High Performance Liquid Chromatography (HPLC) in several preparations of maize. Eight different phenolic compounds from different preparations of maize, namely, gallic, ferulic, vanillic, tannic, caffeic, O-coumaric, cinnamic acids and salicylic acid were observed. Their role in human health is discussed here.

Materials and Methods

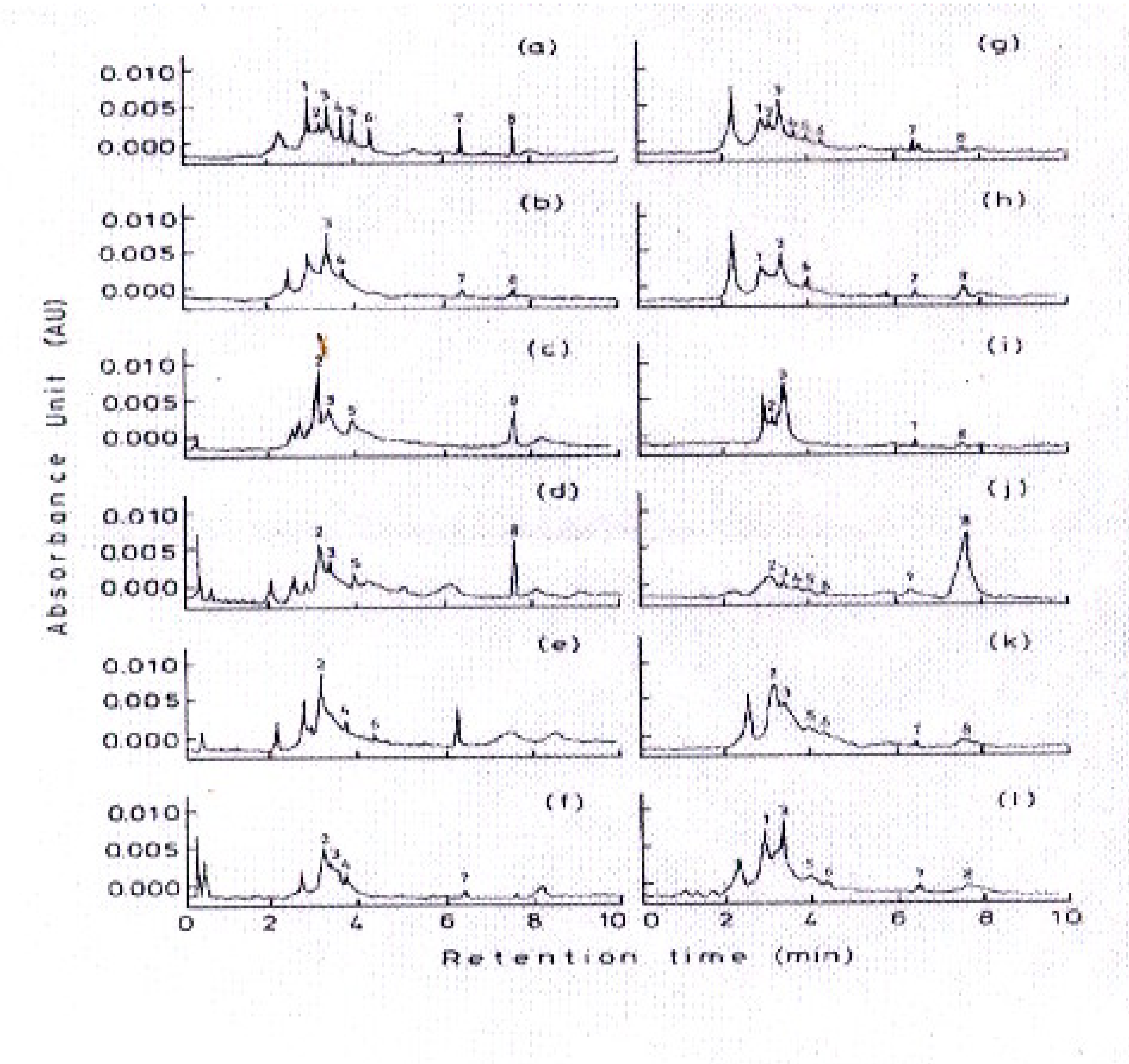
HPLC analysis

Quantitative analysis of the samples was performed as per the method of Singh et al. (2002). The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV- VIS detector and a Rheodyne Model 7725 injector with a loop size of 20 µl. Peak area was calculated with Winchrom integrator. Reverse phase chromatographic analysis was carried out in isocratic conditions using C-18 reverse phase column (250 x 4.6 mm id.) particle size 5 µm (Luna 5 µ (C-18 (2), Phenomenex, USA) at 25^{o}C. Running conditions included injection volume 5 µl, mobile phase methanol: 0.4% acetic acid (80:20 v/v), flow rate 1 ml/min and detection at 290 nm. Samples were filtered through membrane filter (pore size 0.45µm, E-Merck, Germany) prior to injection in sample loop. Tannic, gallic, vanillc, caffeic, ferulic, chlorogenic and cinnamic acids were used as internal and external standards. Phenolic acids present in the sample were identified by comparing chromatographic peaks with the retention time (Rt.) of individual standards and further confirmed by co-injection with standard phenolic acids.

Results and Discussion

HPLC profile of different preparations of maize is shown in Figure. 1 shows several peaks. The entire cob of corn (*Zea mays*) is rich in phenolic acids. Baby cob contained five phenolic acids, namely, gallic (GA), tannic (TA), vanillic (VA), chlorogenic (Chl-A) and caffeic acids (Caff. A) and also salicylic acid. VA was maximum (121.40 µg/g fresh wt.) followed by Caff. A (11.27 µg/g), TA (4.58 µg/g), CA (0.11 µg/g) and SA (0.69 µg/g). In raw corn seed only three phenolic acids were detected among which gallic acid (GA) was maximum (4.6 µg/g) followed by Caff-A (3.2 µg/g), ferulic acid (FA) (0.99 µg/g) and SA (0.57 µg/g). Boiled corn seeds also showed only three phenolic acids where GA was maximum (1.3 µg/g) and others in small amounts such as Caff-A 0.99 µg/g, FA 0.33 µg/g and SA in trace. Boiled corn seeds contained a maximum of 1.30 µg/g. VA, followed by very small amounts of GA (0.20 µg/g) and O-Coum.A in trace (0.003 µg/g). However, four phenolic acids, *viz*., GA (5.37 µg/g), VA (2.17 µg/g), Caff-A (2.99 µg/g) and CA (0.01 µg/g) were

Figure.1 HPLC profile of different phenolic acids present in different preparations of maize.



A-Standard B- Popcorn C- Cornflakes D- Cornflour E- Cobleaves F- Stylar part G -Babycob H- Rawmaize I- Boiled maize seeds J- Baked maize seeds; K- Fried maize seeds L- Maize seed ripe.

1. Tannic acid (TA), 2. Gallic acid (GA), 3. Caffeic acid (CA), 4. Vanillic acid (VA) 5. Ferulic acid (FA), 6. O-coumaric acid (O-CA), 7. Cinnamic acid (CA) 8. Salicylic acid (SA).

found in fried maize seeds. On the contrary, ripe maize seeds showed the presence of seven phenolic acids, viz., TA (2.21 µg/g), GA (0.37 µg/g), VA (1.59 µg/g), O-Coum. A (1.01 µg/g), Caff-A (5.41 µg/g), FA (1.40 µg/g), CA (0.02 µg/g) and SA (0.19 µg/g fresh wt.). Pop corn had only four phenolic acids where TA (2.57 µg/g) was maximum, while O-Coum. A, Caff-A and CA were in small amounts along with SA (0.92 µg/g). Interestingly corn flake revealed the presence of five phenolic acids where GA (18.9 µg/g) was in a very high amount along with Caff-A (4.032 µg/g), TA (8.57 µg/g), O-Coum. A (4.53 µg/g) and CA (0.26 µg/g). SA was 0.96 µg/g fresh wt. Surprisingly, in corn flour, SA content was very high, viz., 13.04 µg/g fresh wt. while other phenolic acids were in small amounts (GA 1.99, VA 0.19, O-Coum. A 0.44, Caff-A 0.54, FA 0.40 and CA 0.05 µg/g fresh wt.). Cob leaves also contained high amount of two of the five phenolic acids i.e., GA 10.90 and Caff-A 6.0 µg/g fresh wt. while others where in small amounts (O-Coum. A 0.66 µg/g, FA 1.87 µg/g, CA 0.04 µg/g fresh wt.). SA was in trace.

Analysis of stylar also showed the presence of some phenolic acids, viz., TA (1.77 µg/g), O-Coum. A (0.97 µg/g), Caff-A in high amount (4.32 µg/g), FA (0.50 µg/g), CA in trace (0.006 µg/g) and SA (0.47 µg/g fresh wt) (Table 1).

The structural formulae of some phenolic acids are shown in Figure. 2. Phenolic acids are found mostly in plants. Animals / human beings do not have any biochemical pathway to synthesize phenolic acids but they need them essentially for different purposes. Some of the phenolic acids have shown pharmacological properties for curing various diseases in human beings (Chung *et al*., 1998; Sharhzad and Bitseh, 1998). Antifungal and antibacterial properties of some phenolic compounds have also been reported in literature (Sarma and Singh 2003; Binutu and Cordell, 2000; Metraux *et al*., 1990; Harborne, 1991). Besides such effects, insecticidal, immuno-stimulating, antioxidant, antimutagenic, anti-inflammatory, allelopathic, disease resistance in onions and apple are the other function of phenolic acids (Barbee *et al*., 1998; Teranchi *et al*., 1997; Metraux *et al*., 1990; Abbas *et al*., 1997; Link *et al*., 1929; Harborne, 1991; Abeysekera *et al*., 1999). (Table 2).

The therapeutic uses of phenolic acids in human health are well known as they act as antioxidants, antidepressant, anti-inflammatory with several other properties. The phenolic profile of different preparations of maize suggests that the quality and quantity of phenolic acids undergo change during different preparations. VA was maximum (121.40 µg/g fresh wt.) in baby cob which is responsible for imparting flavor in its preparation. Similarly, the high amount of Caff-A in corn flakes indicates that it is highly antimicrobial (Graf, 1992). GA is anti-inflammatory and cytotoxic against all cancerous cells (Inoune *et al.*, 1995). The high amount of gallic and ferulic acids in most of the preparations appear to play a crucial role in human health. This phenolic acid was in good amount in raw maize and fried maize seeds, corn flakes and cob leaves. GA and its ethyl ester are the most potent scavengers of super oxide radicals (Fernandes *et al*., 1998), FA as anti-oxidant (Graf, 1992) and antifungal. Caff-A affects plant pathogenic bacteria and fungi (Ravn *et al*., 1989). CA and its derivatives provide natural protection against infections by pathogenic micro organisms. CA affects plasma membrane H+ -ATPase activity of *Saccharomyces cerevisae* (Campbell *et al*., 1999).

Bushman *et al*., (2002) reported that chlorogenic acid in maize sills has been implicated in resistance to corn earworm (*Halicoverpa zea* Boddie). Santiago *et al*., (2005) also expressed their views that phenolic compounds present in pith of 13 imbred maize lines namely, p-Coum. A, Caff-A, FA, VA, SA, CA, sinapic acid, p-hydroxybenzoic acid and vanillin are involved in imparting resistance against stem borer *Sesamia novagrioides* which is a very important insect pest of maize. Similarly, (Sen *et al*., 1994) also studied the effect of phenolic compounds in maize kernels that impart resistance to insect pests. Phenolic acid analysis of corn fiber has also been done by (Yadav *et al*., 2007). Phenolic profiles of Andean purple corn (*Zea mays* L.) has been analysed by (Pedreshci and Cisneros-Zevallos, 2005).

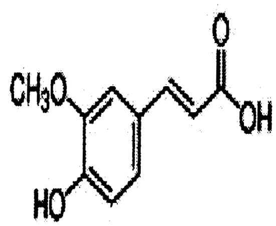
Table.1 Phenolic acids in different preparations of *Zea mays*

Maize Sample	**Phenolic Acid (µg/ml dry wt.)**							
	Tannic Acid	**Gallic acid**	**Vanillic acid**	**O- coumaric acid**	**Caffeic acid**	**Ferulic acid**	**Cinnamic acid**	**Salicylic acid**
Baby cob	4.58	-	121.40	-	11.27	-	0.11	0.69
Raw maize seed	-	4.6	-	-	3.2	0.99	-	0.57
Boiled maize seed	-	1.3	-	-	0.99	0.33	-	0.003
Baked maize seed	-	0.20	1.30	0.003	-	-	-	-
Fried maize seed	-	5.37	2.17	-	2.99	-	0.01	-
Ripe maize seed	2.21	0.37	1.59	1.01	5.41	1.40	0.02	0.19
Pop corn	2.57	-	-	0.59	0.60	-	0.01	0.92
Corn flakes	8.57	18.9	-	4.53	40.32	-	0.26	0.96
Corn flour	-	1.99	0.19	0.44	0.54	0.40	0.05	13.04
Cob leaves	-	10.99	-	0.66	6.0	1.87	0.04	0.0003
Stylar	1.77	-	-	0.97	4.32	0.50	0.006	0.47

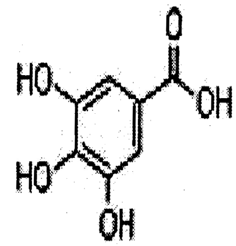
Table.2 Phenolic acids and their biological activity

Phenolic acid	Property	References
Ferulic acid	Antifungal against *Sclerotium rolfsii*	Sarma and Singh, (2003)
Ferulic acid and its dimmers	Catalyzed by peroxidases	Fray, (1986)
Ferulic acid and its dimmers	Allelopathy effect	Harborne,(1991)
Ferulic,chlorogenic, caffeic protocatechuic and p-hydroxybenzoic acid	Immunostimulating properties	Berbec *et al*., (1998)
Quinones and tannins	Insecticidal	Harborne, (1991)
Gallic acid Polyphenol and Gallic acid	Antibacterial Anticonvulsant activity	Binutu and Cordall,(2000) DeLima *et al*., (1998)
Chlorogenic acid	Primary and secondary antioxidant	Lugasi *et al*., (1999)
Chlorogenic acid, quercetin and kaempferol	Potent antioxidative activity	Terauchi *et al*., (1997)
Chlorogenic acid	Antioxidant for low density protein	Donovan *et al*., (1998)
Chlorogenic acid methyl ester	Inhibitory against HIV-protease enzyme which is essential for viral proliferation	Matsuse *et al*., (1997)
Flavonoids and Chlorogenic acid	Antioxidant	Jung *et al*., (1999)
Epicatechin and 4'-caffeorylquinic acid	Developed resistance to scab *Venturia inaequalis*	Metraux *et al*., (1990)
Plant polyphenols (tannins)	Biological antioxidants	Hagerman *et al*., (1998)
Flavanols	Antimutagenic	Abbas *et al*., (1997)
Gallic acid and hudroquinone	Antityrosinase activity	Matsuo *et al*., (1997)
Gallic and Salicylic acid	Important role in insect-plant interaction	Ananthakrishan, (1997)
Protocatechuic acid	Disease resistance in onions	Link *et al*., (1929)
Protocatechuic acid	Fungicidal activity	Harborne, (1991)
Luteolin, Chlorogenic, diffeorylquinic acids	Anti-inflammatory	Abeysekera *et al*., (1999)
Hydroxycinnamic acid (Sialic acid)	Allelopathy effect	Harborne, (1991)

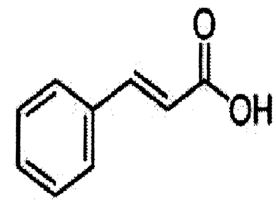
Figure.2 Chemical structure of different phenolic acids.



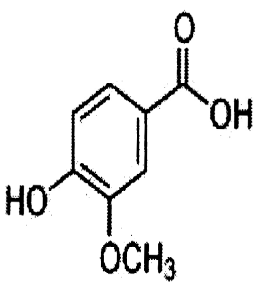
Ferulic acid



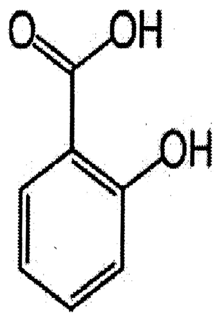
Gallic acid (3,4,5-trihydroxybenzoic acid)



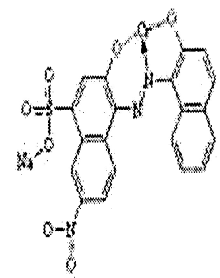
Cinnamic acid



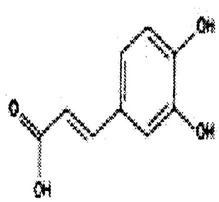
Vanillic acid
(4-hydroxy-3-methoxybenzoic acid)



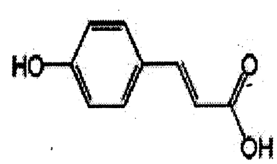
Salicylic acid



Tannic acid



Caffecic acid



O-Coumaric acid

High Performance Liquid Chromatographic analysis revealed the presence of p-coumaric, vanillic, protocatechuic acids and flavonoids such as quercetin derivatives and a hesperitin derivative in ethyl acetate fraction (EAF). Alkaline and acid hydrolysis of the EAF shoed p-coumaric and ferulic acids as main component. Since all the maize preparations contain several phenolic acids and some of them in good amount their consumption is always in favor of good human health. Results of the present study support preferential use of corn flakes for better human health. Seeing the high amount of some phenolic acids needed to import resistance, to ward off several diseases and fulfill several nutritional requirements it is suggested that maize should be included as an important component in human diet. However, review of literature indicates that no detailed study has been done so far on phenolic acid contents in different edible preparations of maize. Hence, our results form the first report in this regard.

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