International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 20-08-2021, Accepted: 05-09-2021, Published: 20-09-2021
Volume 6, Issue 5, 2021, Page No. 48-55

Maize bran: A promising source of ferulic acid-rich dietary fibre for making functional cookies

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Abstract
Ferulic acid (FA), present in cereal brans as part of dietary fibre has considerable antioxidant and antityrosinase activities. In the present work, FA was chemically extracted from bran of different hybrids of yellow maize. The highest FA was recorded in bran from Bio9681 maize and hence was used in the formulation of composite flours, wherein refined flour was replaced by maize bran @ 0, 10, 25, and 50% concentrations to prepare FA-rich cookies. FA from the bran of Bio9681 maize also showed considerable antioxidant (69. 05 µMVCEAC mg/g) and antityrosinase activities (14. 05 µM KAE). The formulated cookies and their respective batters were analyzed for FA (free and total) as well as antioxidant and antityrosinase activities. It was observed that no FA was present in control cookies, while it increased with higher substitution of maize bran in the cookies made from composite flours. In addition, the baking process increased the amount of free FA in cookies. Likewise, cookies made from composite flours had higher antioxidant (9. 43-28. 05 µMVCEAC mg/g) and antityrosinase activities (1. 28-4. 08 µM KAE) compared to the control cookies. Furthermore, proximate analysis of cookies proved that corn bran enriched cookies were rich in dietary fiber and ash content compared to control cookies. Finally, the sensory evaluation showed that cookies made from 25% maize bran and 75% wheat flour has maximum sensory attributes. Hence, maize bran is a good source of FA and may be recommended for the preparation of nutritionally improved functional cookies by replacing 25% wheat flour with maize bran.

Keywords: antioxidant, antityrosinase, cereal bran, ferulic acid, sensory evaluation

Introduction

Diet and nutrition play an important role in supporting a healthy life which also prevents various chronic diseases. Unhealthy food choices lead to obesity and other associated diseases, thereby increasing healthcare costs. In this respect, the health benefits of dietary fibre are well recognized as increased consumption help in regular laxation, lower the risks of cardiovascular disease, type2 diabetes, colorectal and other types of cancers, and very importantly, is inversely associated with body weight as it increases satiation (Howarth *et al.*, 2009)[20]. Despite all these known health benefits of fibre-rich foods, the average daily intake of fibre is still lower than that recommended. Nevertheless, cereals are one of the richest sources of dietary fibre, as a result, fortification of cookies, bread, and other food products with cereal brans can provide high fibre nutraceutical foods to the population. Dietary fibre mainly includes arabinoxylans, which comprises a linear (1–4)-β-D-xylopyranose chain that is substituted with L-arabinofuranose at O-2 and/or O-3 position. The arabinoxylans (AX) may be feruloylated with ferulic acid (FA) at the O-5 position of the arabinose units (Bunzel *et al.*, 2006) [9]. The biological properties of AX include prebiotic, antioxidant, and anticancerous properties. However, these properties of AX depend on its structural characteristics and it has been well established that the presence and appearance of FA in AX directly affect its antioxidant and prebiotic properties (Snelders *et al.*, 2014) [33].

Among cereals, FA content is highest in maize and is also the predominant phenolic acid present in maize. Recently, Malunga and Beta (2016) [25] reported 2. 5 times higher esterified ferulic acid (FA) in maize compared to wheat. On the other hand, the FA content of maize also varies depending on cultivars, and the processing conditions (Sidhu *et al.*, 2007) [32]. Further, in maize, most of its FA is present in the pericarp (i. e. maize bran), while it is negligible in germ and endoplasm (Chateigner-Boutin *et al.*, 2016) [11], making maize bran a rich and potential source of FA. Furthermore, since maize bran is a by-product of the cereal milling industry, which is either discarded or used as animal feed, its utilization will make the production process cost-effective and also aid in solving the problem of agro-waste disposal to some extent.

FA is also a potent antioxidant, known to inhibit oxidation of biomolecules by reducing the reactive oxygen species levels, thus preventing damage caused by free radicals in body cells (Alam, 2019) [4]. Moreover, the presence of FA in food items can preserve the oils present in the bakery goods from undergoing oxidation and provide antioxidant effects to the body. Besides, the structural similarity of FA with tyrosine, it has potential antityrosinase activity, inhibiting the enzyme through competitive inhibition (Yu *et al.*, 2019) [36]. Studies have demonstrated that tyrosinase had a primary role in the enzymatic browning reaction in foods during processing by catalyzing the oxidation of phenols (Lin *et al.*, 2011) [22]. Tyrosinase is also a key enzyme of melanogenesis, whose overproduction can lead to skin carcinomas (Sun *et al.*, 2017) [34]. As a result, FA in food products may help in the prevention of melanin hyperpigmentation to some extent and also increase the shelf life of the food products by inhibiting tyrosinase catalyzed browning. Although FA is an FDA-approved preservative, its direct addition to functional foods has the drawbacks of photodegradation and sensitivity to oxygen exposure (Aceituno-Medina *et al.*, 2015) [3]. Alternatively, FA-rich cereal brans can be directly used as an ingredient in a variety of food products, especially bakery products such as cookies and bread to get health benefits as well as a reduction in browning.

Based on the available information, the present study has been undertaken to explore the value-addition of maize bran as a source of ferulic acid. The FA content in the bran of different hybrids of yellow maize was estimated and also checked for antioxidant and antityrosinase activities. The bran from the highest FA containing maize hybrid was used for making composite flours in which refined wheat flour was replaced by maize bran at 0%, 10%, 25%, and 50% concentrations. The cookies made were evaluated for proximate composition, free and total FA content, antioxidant and antityrosinase activities as well as sensory characteristics. The study also aimed to investigate changes that occurred in the amounts of free and bound FA content present in maize bran during baking.

Materials and methods

Raw material and chemicals

Different yellow maize hybrids cultivated in India viz. Bio 9681, HQPM5, CMH08-292, SeedTech2324, Vivek QPM9 were procured from the Division of Genetics, IARI, New Delhi, India. HQPM5 is high-quality protein maize and Vivek QPM9 is early maturing quality protein maize. Seed Tech 2324 is adaptive to changing climate conditions and CMH08-292 is an early shelling maize hybrid. On the other hand, Bio 9681 is identified as a high seed quality maize. The pedigree details of the tested hybrids are given in supplementary Table 1. Refined flour, sugar, and oil were brought from the local market. Trans-ferulic acid was purchased from Merck, USA. Other chemicals were supplied from SRL Pvt. Ltd, Mumbai, India.

Table 1: Proximate composition of formulated cookies

Cookie sample	Moisture (%)	Ash (%)	Crude fiber (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Control (T0) - Made from wheat flour	3.71 ± 0.09^{a}	0.50 ± 0.03^{a}	0.32 ± 0.05^{a}	20.71 ± 0.66^{a}	11.83 ± 0.27a	63.58 ±1.27^{a}
T1- (10% maize bran substitution)	4.21 ± 0.15^{b}	0.72 ± 0.03^{b}	1.22 ± 0.08^{b}	19.76 ± 0.38^{ab}	12.17 ± 0.25^{a}	61.70 ± 0.46^{b}
T2- (25% maize bran substitution)	4.69 ± 0.13^{c}	1.41 ± 0.05^{c}	3.31 ± 0.19^{c}	19.43 ± 0.53^{b}	12.88 ± 0.19^{b}	58.24 ± 0.62^{c}
T3- (50% maize bran substitution)	4.94 ± 0.15^{d}	1.51 ± 0.06^{d}	5.52 ± 0.39^{d}	18.80 ± 0.69^{b}	13.08 ± 0.38^{b}	55.78 ±0.80^{d}

$^{a-d}$ Values in the same column with different alphabets (superscript) are significant and values with the same alphabets (superscript) are not significant, $P< 0.05$

Preparation of substrate

Maize grains of all the hybrids used were ground separately in a grinder to obtain maize flour which was sieved from a 1 mm sieve to separate the bran. The separated brans were dried and stored in airtight containers.

Alkaline extraction of ferulic acid from maize bran

Ferulic acid was extracted from the bran of each maize hybrid used in the study by the method described by Aarabi *et al.* (2016) [1]. Maize bran (0. 1 g) was defatted with hexane, centrifuged (7000 × g, 10 min), and hydrolyzed with 6 ml of 0. 5 N KOH and incubated at 50°C for 18 h at continuous stirring. The alkaline hydrolysates were acidified (pH <2) with dilute HCl (2M) to protonate the phenolic acids, rendering them extractable into an organic solvent and also to precipitate hemicelluloses. The mixture was centrifuged at 10, 000 rpm for 20 min and the supernatants were collected. The released FA in the supernatant was extracted twice using equal volumes of diethyl ether. Further, the ether extracts were extracted with $NaHCO_3$ solution (5%). This step extracts phenolic acids into the aqueous layer; other phenolic compounds mainly remain in the organic layer and are discarded (Dobberstein and Bunzel, 2010) [14]. The aqueous layers were acidified (pH < 2), and phenolic acids were re-extracted into diethyl ether. Ether extracts were dried over Na_2SO_4, evaporated to dryness, and redissolved in 80% methanol. The amount of FA extracted was quantified using HPLC as described below.

HPLC based estimation of ferulic acid

Estimation of FA in all the samples was performed by high-performance liquid chromatography (HPLC) system (Waters 515 pump), fitted with a Zorbax C18 column (300 × 7. 8mm) coupled with a PDA detector 2998 at 310 nm and autosampler 2707. The injection volume was 10 µl and elution was carried out isocratically at 0. 5 ml/min flow rate, using a mobile phase composed of methanol/0. 3% acetic acid (35:65). The quantification of ferulic acid in maize bran extracts was accomplished by using the standard curves made from different dilutions of ferulic acid from Merck.

Formulation of cookies

Cookies were prepared according to AOAC method-10-50D (2000). According to which, 50 g of wheat flour, 25 g sugar, 25 g shortening, 1 g $NaHCO_3$, and 1 g NaCl were mixed in a bowl to form the batter. This composition of the flour was used for making control cookies (T0). While, test cookies were prepared by composite flours, in which wheat flour was replaced with maize bran @ 10% (T1), 25% (T2), and 50% (T3) concentrations, keeping the other ingredients constant. The batters formed were shaped into respective cookies and baked in an electric oven at 180°C for 10 min. Baked cookies were allowed to cool and then stored in airtight containers for further analysis.

Proximate analysis of cookies

Proximate analysis of the cookies was performed according to the standard methods of AOAC (Horwitz, 2000). Samples were analyzed for moisture using ACZET MB-50 Moisture Analyzer. The crude fat analysis was done using the Soxhlet Extraction Method. Fibre analysis was carried out using Fibretech^{TM} 8000 Fibre analyzer. Ash content was quantified by the dry ashing method. Crude protein content was estimated by the Micro-Kjeldahl Method. The carbohydrate content in cookies was calculated using the following equation:

% carbohydrates= 100 – [weight in grams (moisture+ protein + fat + ash + fibre) in 100 g of food]

Sample extraction for determination of antioxidant and antityrosinase activities of formulated cookies and their batter

Samples of cookies were ground into fine particles while the batter was taken as such. Samples (1 g each) were mixed with 100 ml methanol (80% v/v) in conical flasks (250 ml) and shaken overnight at 150 rpm and 30°C. The mixtures were centrifuged at 3000 rpm for 30 minutes and the supernatants obtained were used for estimation of antioxidant and antityrosinase activities.

Sample extraction for determination of free and total FA in cookies and their batter

For the determination of free FA, 50 mg of ground cookie and batter was mixed separately with 2 ml water and HCl (pH <1. 5). The mixtures were extracted twice with diethyl ether and evaporated to dryness. The dried extracts were redissolved in 0. 5 ml of 80% methanol and filtered through a 0. 45µ filter before injection into HPLC for FA estimation. For the determination of total FA, the samples were extracted using the alkaline extraction method as described in the previous section and FA content was estimated by the HPLC method.

Determination of antioxidant activity

The antioxidant activity of samples was determined by the improved $ABTS^{\cdot +}$ decolorization assay as described by Re *et al*. (1999). A standard curve was prepared by using different concentrations of Vitamin C (20-200 µg/ml) and the $ABTS^{\cdot +}$ scavenging activity of all the extracts was expressed as µM of Vitamin C equivalent antioxidant capacity (VCEAC).

Determination of antityrosinase activity

The antityrosinase assay was performed following the method given by Pintus *et al*., (2015) [29]. A standard curve was prepared by using different concentrations of kojic acid (5-50µg/ml) and the antityrosinase activity of the extracts was expressed as µM of kojic acid equivalent antityrosinase capacity (KAEAC).

Sensory evaluation

A panel of thirty members comprising of staff and students (in the age group 20-50 years) evaluated the sensory properties of the cookies. The panel members were directed to give ratings for each sensory characteristic (color, appearance, flavor, crunchiness, texture, and taste) using the control cookies as a reference on a 9-point hedonic scale. (9-like extremely, 8- like very much, 7-like moderately, 6- like slightly, 5- neither like nor dislike, 3-dislike moderately, 2-dislike very much, 1-dislike extremely). The overall acceptability was determined based on quality scores obtained from the evaluation of color, flavor, crunchiness, and texture of the cookies. Water was used to rinse the mouth between evaluations. The samples were coded with alphabets and given to the panelists randomly to prevent any bias.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of triplicate measurements. The data was subjected to one-way analysis of variance to determine significant differences between them using Minitab Software (version 12, Minitab Inc., State College, PA). Differences were considered to be significant when p<0. 05.

Results and Discussion

Estimation of FA in maize bran samples

HPLC analyses revealed that the retention time (RT) of standard ferulic acid (FA) from Merck under the given conditions was 9. 75 min. The FA extracts from the bran of different maize hybrids *viz*. Bio 9681, Seedtech2324, HQPM5, Vivek QPM9, and CMH08-292 showed corresponding FA peaks in HPLC as detected with the standard FA (supplementary Fig. 1). However, significant differences in the FA concentration were observed in the bran of the tested maize hybrids. The maize bran of Bio 9681 was found to contain the highest amount of FA (4. 5%), followed by SeedTech2324 (3. 1%), HQPM5 (1. 8%), Vivek QPM9 (1. 0%), and CMH08-292 (0. 7%). The FA content of maize bran has been reported in the range of 2. 61-3. 30% (Zhao *et al.*, 2005) [37]. In the present study, the difference in FA content indicates significant genotypic differences in the tested maize hybrids. Similar to our results, Ayala-Soto *et al*. (2014) [7] also reported differences in the hydroxyl cinnamic acid content in maize fibre from different sources. Thus, it can be inferred that FA content varies in different maize cultivars and is also dependent on the processing conditions (Sidhu *et al.*, 2007) [32].

Increased FA in some maize hybrids has been explained by some researchers as an adaptation to the existing climatic conditions (Hura *et al.*, 2008) [21]. An increase in FA production in drought-tolerant maize hybrids was observed when compared with single-cross hybrids by Hura *et al.* (2008) [21]. The authors concluded that increased formation of reactive oxygen species under drought (stress) conditions stimulate/induce the production of FA, which is a known antioxidant. In this respect, Mahajan and co-workers, (2012) [23] found Bio 9681 and Seed Tech 2324 varieties of maize are more adaptive to change in climate/rainfall, compared to the other tested maize varieties. Thus, it can be postulated that increased production of FA/ phenolics under stress conditions in some maize hybrids helps the plant to reduce oxidative stress and thus, survive better during climate change. While, Guo and Beta (2013) [18] identified and quantified various phenolic acids from the alkaline extracts of eight kinds of cereal (barley, purple barley, wheat, purple wheat, red rice, yellow corn, and oats) and found out ferulic acid as the most abundant one in all the extracts with highest present in the insoluble dietary fibre extracts of yellow corn (2-10 more than the alkaline extracts of other cereals tested). Recently, Cheng *et al.* (2018) [13] observed that FA pretreatment increased the transcription of genes encoding iron superoxide dismutase, cytoplasmic copper/zinc superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase and an elevated content of proline and soluble sugars in blueberry leaves, thereby alleviating heat stress to the plant.

Proximate composition of formulated cookies

Table 1 summarizes the proximate composition of the control and test cookies. Moisture content is a critical parameter as far as the texture, acceptability, and shelf life of the cookies are concerned. In the present work, the moisture content in cookies ranged between 3. 8-5. 0%, which is within the recommended range of 0-10% for the storage of cookies. As can be seen from Table 1, the moisture content of the cookies increased with an increase of maize bran in the formulation. This may be due to the higher water absorption and water holding capacity of maize bran fibre. It is a known fact that the moisture content of food products increases with the fibre content (Uthumporn *et al.*, 2015) [35]. Whereas the fat content of the cookies decreased slightly and protein content increased slightly as wheat flour was replaced with maize bran. On the other hand, the ash content of the cookies made from blends of maize bran and wheat flour was significantly higher than the cookies made solely from wheat flour. Ash content is an estimate of the total mineral content in a given quantity of food substance. The increase in mineral content could be due to the maize bran fraction of the blended flours as maize bran has higher mineral content as compared to wheat flour (Pauline *et al.*, 2020) [28]. Similarly, the dietary fibre content also increased significantly in the cookies when wheat flour was replaced with maize bran. Control wheat biscuits had the lowest crude fibre content of 0. 32% ± 0. 05 and incorporation of maize bran at 10% (T1), 25% (T2) and 50% (T3) levels had significantly increased the fibre content to 1. 22% ± 0. 08, 3. 31% ± 0. 19 and 5. 52%± 0. 39, respectively. This is because dietary fibre is concentrated in the seed pericarp (bran) and among cereal brans, maize bran is characterized by a high content of dietary fibre and phenolics. Paraskevopoulou *et al.* (2019) [27] observed a 4 to 6-fold increase in fibre content of wheat flour biscuits when substituted with maize bran @ 10 and 20% concentrations.

Further, the carbohydrate content of the cookie samples ranged between 55. 78% to 63. 58% with the highest in control cookies followed by T1, T2, and T3 cookies. Generally, enriched foods have lower carbohydrate content than control. These results can be explained by the fact that cereal brans have a low content of simple sugars but are richer in complex sugars including fibre. Pauline *et al.* (2010) also demonstrated that carbohydrate content decreased from 85. 03% to 80. 38%, when wheat flour was gradually substituted with maize bran in bread.

FA content of cookies and their batters

On determining the FA content of the cookies and their respective batters as shown in Table 2, it was observed that no FA was present in the control cookie (T0) as well as in its batter. While, in the cookies and the batters made from different flour blends of maize bran and wheat flour, the amount of FA increased with percent incorporation of maize bran. The highest amount of % bound FA content (1. 60 ± 0. 07) was observed in a batter made for T3 cookies with 50% substitution of wheat bran. At the same time, the amounts of bound FA decreased slightly in the cookies compared to their respective batters (Table 2). Different maize bran substituted batters also had small amounts of free FA (%), with the highest in T3 (0. 06 ± 0. 007) followed by T2 (0. 05 ± 0. 008) and T1 (0. 04 ± 0. 01). However, the amount of free FA increased substantially in the cookies made from these blends (0. 51± 0. 03 in T3, 0. 25 ± 0. 04 in T2, and 0. 14 ± 0. 02 in T1). The apparent increase in free FA is due to the release of bound FA during baking which may also be responsible for a slight reduction in its bound form. It has been reported that heat stress could degrade conjugated polyphenolic compounds increasing free phenolic acids in cereals (Cheng *et al.*, 2006) [12]. This would improve the bioavailability of phenolic compounds in the body since free phenolic acids are more readily available than bound phenolic compounds. In general, baking is an efficient way to improve the bioavailability of phenolic acids in whole grain products by the release of bound phenolic acid (Abdel-Aal and Rabalski, 2013) [2]. The current study showed that the baking process may liberate the bound ferulic acid present in the maize bran and thereby increase the free ferulic acid content in the cookies made from it. Similar results have been reported by Cheng *et al.* (2006) [12] who found that heat stress could cause degradation of conjugated polyphenolic compounds, increasing free phenolic acids in wheat.

Interestingly, Abdel-Aal and Rabalaski (2013) [2] also observed that baking was responsible for an increase in free FA content in whole grain muffins, bread, and cookies vs whole grain flour.

Table 2: Free and total FA content of cookies and their respective batters

Sample	% FA			
	Batter		Cookie	
	Free	Bound	Free	Bound
Control (100% WF)	ND	ND	ND	ND
WF: MB (90:10) T1	0.04 ± 0.01^{b}	0.31 ± 0.02^{c}	0.14=5± 0.02^{c}	0.27 ± 0.02^{c}
WF: MB (75:25) T2	0.05 ± 0.008^{ab}	0.80 ± 0.034^{b}	0.25 ± 0.04^{b}	0.75 ± 0.04^{b}
WF: MB (50:50) T3	0.06 ± 0.007^{a}	1.60 ± 0.07^{a}	0.51 ± 0.03^{a}	1.35 ± 0.11^{a}

ND- not detected, WF- wheat flour, MB- maize bran

$^{a-c}$ Values in the same column with different alphabets (superscript) are significant and values with the same alphabets (superscript) are not significant, $P< 0.05$

Determination of antioxidant activities

The purified FA extract from the bran of Bio 9681 maize hybrid when tested for $ABTS^{\cdot +}$ scavenging capacity, the recorded activity of 69. 05 ± 2. 89 μMVCEAC. Similarly, when cookies amended with maize bran were analyzed for their antioxidant capacity, the antioxidant activity increased with percent amendment, and the highest $ABTS^{\cdot +}$ radical scavenging capacity of 20. 69 ± 0. 80 μMVCEAC and 28. 64 ± 0. 77 μMVCEAC was recorded in T3 batter and cookies respectively (Table 3). This may be correlated with the increase in the FA content of the cookies by the incorporation of maize bran in their flour blends. Ferulic acid is reported to be a major contributor to the antioxidant activities of cereal grains (Alam, 2019) [4]. Guo and Beta (2013) [18] reported corn insoluble dietary fibre (IDF) extracts with higher levels of total phenolic content, ferulic acid content, and antioxidant activity (measured by DPPH radical scavenging activity) compared to other cereals tested (barley, wheat, rice, and oats). Further, the correlation analyses between phenolic acids and the antioxidant potential of cereal grains suggested that the phenolic acids are the main compounds responsible for the antioxidant potential of cereal alkaline extracts. The authors thus recommended corn as a potential antioxidant value-added functional food ingredient (Gou and Beta, 2013) [18]. Bento silva *et al.* (2020) identified hydroxycinnamic acids in maize flour and Broa (traditional ethnic maize bread) and observed ferulic acid as the major phenolic acid in both soluble and insoluble fractions of maize flours and *broas*. The authors also concluded that since the hydroxycinnamic derivatives were not only identified in maize flours but also *broas*, they can contribute to the antioxidant properties and beneficial health effects of maize-based foods.

Further, it was also observed that the antioxidant activities of cookies were higher than their respective batters (Table 3). This can also be attributed to the increase in the free FA content of the cookies during baking as discussed in the previous section. In this regard, it has also been reported that Maillard reaction products formed during baking also contribute to the antioxidant activity of the baked product. In a study by Abdel-Aal and Rabalski (2013) [2], it was found that the baking process improved the antioxidant properties of whole grain bread, cookie, and muffin. Similarly, Mahloko *et al.* (2019) [24] observed that baking increased the antioxidant activities of wheat flour and banana peel flour cookies. However, these indirect assays like $ABTS^{\cdot +}$ or $DPPH^{+}$ radical scavenging assays for determining the antioxidant potential of food products provide values for the single-electron-transfer antioxidant activity of products, the translatability of these values to biological systems is limited (Arts *et al.*, 2003) [6]. On the other hand, when pure molecules have to be tested as antioxidants or redox-active drugs, it is very important to achieve mechanistic insights that will enable drawing structure-activity relationships (Amorati and Valgimigli, 2015) [5]. In these cases, inhibited autoxidation studies are the most valuable as single-point measurements of secondary oxidation products might serve as preliminary tests (Bunzel and Schendel, 2017). In addition, due to their simplicity, the indirect assays (e. g DPPH or ABTS) might be useful to screen the antioxidant content of natural extracts or matrices of unknown composition (Amorati and Valgimigli, 2015) [5]. However, overestimation of antioxidant content is a likely possibility (*e. g.* by exchanging a highly oxidizable or reducing compound for a real antioxidant) and these assays cannot replace the full analytical characterization of the extract. Therefore, further research on the antioxidant potential of our formulated cookies in cultured human intestinal cell lines is being conducted.

Table 3: Antioxidant and antityrosinase activities of formulated cookies and their respective batters

Sample	Antioxidant activity (µMVCEAC mg/g)		Antityrosinase activity (µM Kojic acid equivalents)	
	Batter	**Cookies**	**Batter**	**Cookies**
Control (100% WF)	2.27±0.10^{a}	3.39±0.21^{a}	ND	ND
WF: MB (90:10) T1	7.58±0.41^{b}	9.31±0.36^{b}	0.83±0.04^{b}	1.28±0.04^{b}
WF: MB (75:25) T2	12.68±0.61^{c}	16.52±0.54^{c}	1.77±0.03^{c}	2.28±0.22^{c}
WF: MB (50:50) T3	20.69±0.80^{d}	28.64±0.77^{d}	3.16±0.12^{d}	4.49±0.31^{d}

ND- not detected, WF- wheat flour, MB- maize bran

$^{a-d}$ values in the same column with different alphabets (superscript) are significant and values with the same alphabets (superscript) are not significant, P< 0.05

Determination of antityrosinase activities

The purified FA extracts from the bran of Bio 9681 maize hybrid showed antityrosinase activity of 14. 05 ± 0. 35 µM KAE (Table 3). Evaluation of antityrosinase activities of the formulated cookies (Table 3), revealed no antityrosinase activity in control cookies. On the other hand, the cookies made from flour blends of wheat flour and maize bran showed antityrosinase activities ranging between 1. 28-4. 49 µM KAE. It was also observed that the antityrosinase activity improved due to blending of maize bran in the flour blends with the highest (4. 49 ± 0. 31 µM KAE) being observed in cookies made from flour composition of WF: MB 50:50. As for antioxidant activity, this is attributed to the increased ferulic acid content of the maize bran cookies compared to the control cookies, which were not found to contain any amount of FA (Table 2). FA is a known antityrosinase compound due to its structural similarity with tyrosinase (Yu *et al.*, 2019) [36]. Tyrosinase is responsible for enzymatic browning in food products by catalyzing hydroxylation of monophenol and oxidation of o-diphenols to o-quinones (Lin *et al.*, 2011) [22]. Additionally, quinones reacted with amines, amino acids, peptides, and proteins could result in a loss of nutritional quality, a decrease of digestibility, and inhibition of proteolytic and glycolytic enzymes. Moreover, tyrosinase is also responsible for melanin distribution in animals, whose excessive production can lead to skin carcinomas (Lin *et al.*, 2011) [22]. Therefore, FA can serve as a natural tyrosinase inhibitor, which can be beneficial for inhibiting enzymatic browning in food products and preventing pigmentation disorders in human beings (Lin *et al.*, 2011) [22]. However, in-vivo studies to confirm any effect of the formulated cookies on melanin hyper pigmentation need to be investigated in future studies. Georgiev *et al.* (2013) [16] also showed high antityrosinase activity of various hydroxycinnamic acids. Further, as observed for antioxidant activities, the antityrosinase activities were also higher in the cookies as compared to their respective batters (Table 3). This is also due to the increased free FA content of the cookies after baking as discussed in the previous section, which contributed to the increase in their antityrosinase activities.

Sensory evaluation of the cookies

It can be seen from Table 4 that there was no significant difference in the overall acceptability of all the cookies. However, differences between different cookies for sensory attributes like taste, color, flavor, and overall acceptability were observed. The sensory rating of the cookie for color shows that the control cookie (8. 45) ranked at the top due to excellent appearance, followed by T1 (8. 10) and T2 (7. 80) while minimum color was observed in T3 (7. 05). With an increasing level of corn bran substitution, the color of the biscuit turned from light brown to dark brown. A decrease in lightness and an increase in brown/redness could be due to added maize bran color and fibre content (Gómez *et al.*, 2010) [17]. Ertas (2015) also observed that wheat flour cookie samples with added wheat bran were darker in color than the control cookie sample with no added wheat bran. The Mean score of taste decreased from 8. 60 to 7. 55 with an increasing level of substitution. While the mean score for crunchiness was highest in T2 (8. 60 ± 0. 31) followed by T1 (8. 05 ± 0. 36) and was statistically same in 100% wheat flour cookies (7. 0 ± 0. 47) as well as in T3 cookies (7. 0 ± 0. 40). The fibre present in maize bran imparted crunchiness to the cookies (T1 and T2) compared to the control cookies, while a lower crunchiness score was observed for T3 cookies. This is due to the high moisture content in T3 cookies, which in turn is due to the higher fibre of maize bran in it. This resulted in T3 cookies getting a bit soggier. Similarly, Muyanja *et al.* (2020) [26] also used maize germ and bran for value addition of confectionary products, wherein wheat flour was replaced by maize bran in the range of 4 to 15% and maize germ in the range of 1 to 10%. The authors observed that the incorporation of 40% for maize bran and 20% for germ resulted in cakes and cookies with good eating qualities. Further, the authors also observed that the cookies made by incorporating maize bran resulted in crunchiness as some of the bran crystals were being tasted in the final product. The mean score for the flavor of biscuit decreased from 8. 45 to 7. 60 with an increasing level of substitution. This could be due to the bland taste of maize bran. The mean score of texture also decreased from 8. 60 to 7. 05 with an increasing level of substitution. Higher substitution of maize bran in cookies made their texture rough compared to the control cookies. However, the mean regarding the overall acceptability of cookies revealed that all the cookies are well accepted by the panel. The overall acceptability of control biscuits was almost the same as that of T2, while T3 has the lowest acceptability. Thus, the optimum substitution levels of maize bran in wheat flour for making cookies is 25%, above this, the product becomes less acceptable to the consumer. Sandhu *et al.* (2017) used corn fibre at 20% substitution of wheat flour to prepare high-quality biscuits with desirable Physico-chemical and sensory properties.

Table 4: Sensory evaluation of formulated cookies

Cookie made from	Wheat flour T0	WF: MB (90:10) T1	WF: MB (75:25) T2	WF: MB (50:50) T3
Color	8.45 ± 0.44^{a}	8.10 ± 0.31^{ab}	7.80 ± 0.35^{b}	7.05 ± 0.44^{c}
Taste	8.60 ± 0.40^{a}	8.35 ± 0.53^{a}	8.10 ± 0.52^{ab}	7.55 ± 0.59^{b}
Crunchiness	7.00 ± 0.47^{c}	8.05 ± 0.36^{b}	8.60 ± 0.31^{a}	7.00 ± 0.40^{c}
Flavor	8.45 ± 0.36^{a}	8.30 ± 0.42^{a}	8.10 ± 0.39^{a}	7.60 ± 0.39^{b}
Texture	8.60 ± 0.39^{a}	8.05 ± 0.55^{ab}	7.60 ± 0.65^{bc}	7.05 ± 0.64^{c}
Overall acceptability	8.21 ±0.68^{a}	8.23 ±0.16^{a}	8.07 ±0.36^{a}	7.28 ± 0.28^{b}

WF- wheat flour, MB- maize bran

$^{a-c}$ values in the same row with different alphabets (superscript) are significant and values with the same alphabets (superscript) are not significant, P< 0.05

Figure Legend

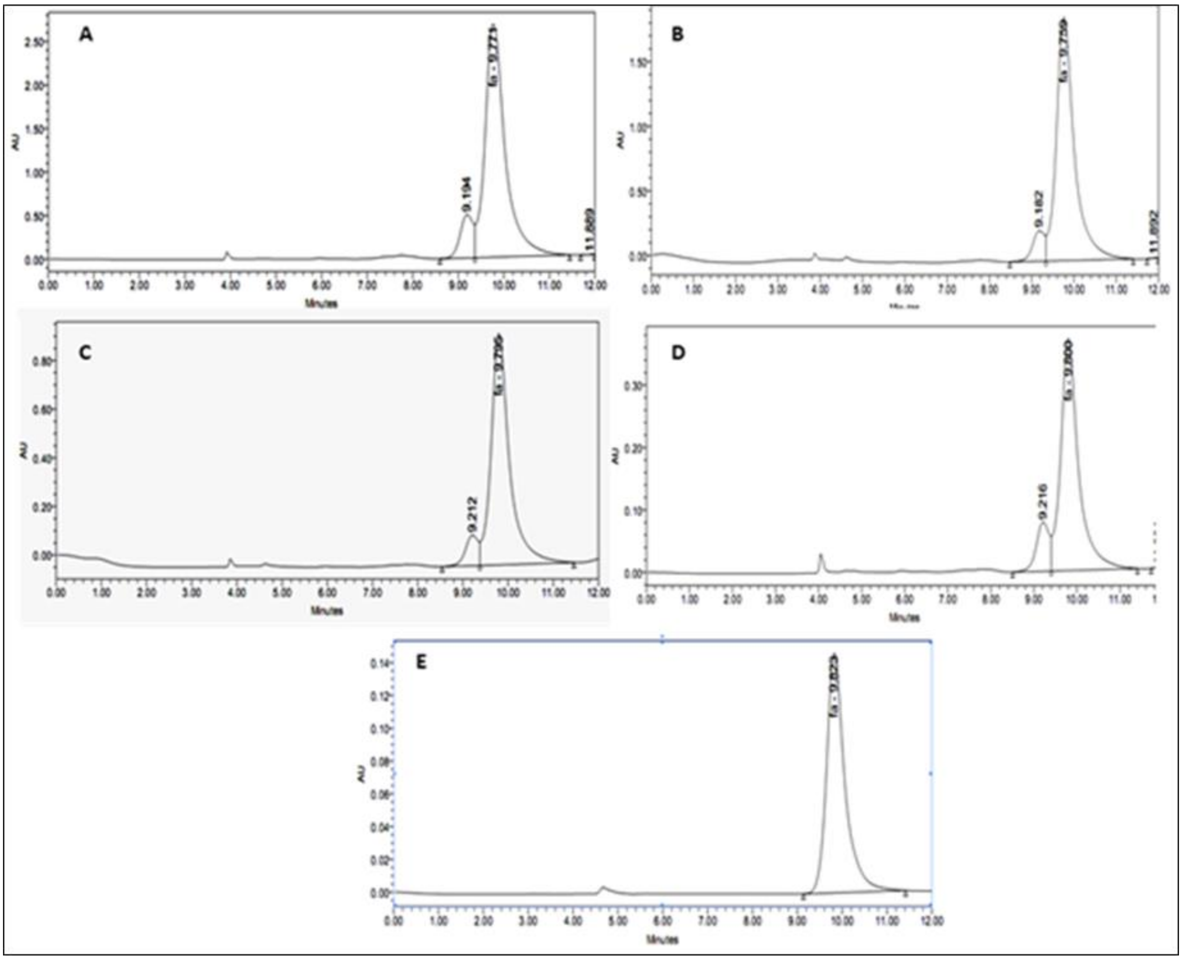

Fig 1: Ferulic acid content in bran of yellow maize A) Bio 9681, B) SeedTech 2324, C) HQPM5, D) Vivek QPM 9, E) CMH 08-292

Conclusion

There is an increasing interest to find new sources of dietary fibre with specific bioactive activities that may add new healthy properties to the traditionally commercialized food products. Ferulic acid could be used as a suitable source of dietary fibre with associated functional properties viz. antioxidant and antityrosinase. The phenolic acid is mainly present in cereal brans, the highest being in maize bran. However, the amount present varies within cultivars depending on the genotype as well as processing conditions. In the present study, FA content in the bran showed intrinsic diversity among the five different hybrid varieties of maize with the highest being observed in Bio 9681 maize. The bran from this maize hybrid was used for making cookies and it was evident from the experiments that cookies can be made with substitution of maize bran up to 25 % in wheat flour without adversely affecting the sensory characteristic of the cookies. These functional cookies are nutritionally more superior to wheat flour cookies in terms of high fibre and ash content as well as increased antioxidant and antityrosinase activities. Maize bran can be a valuable option for nutritional improvement of bakery products, such as cookies which are a popular and widely accepted constituent of diet, among consumers worldwide.

Conflict of Interest Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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