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Characteristics of germinated corn flour and influence of germination on cookie properties

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ABSTRACT

The aim of this study was to investigate the effect of germination on the physicochemical, structure, pasting, and morphological properties of corn. Germination improved total phenolic content (TPC) and antioxidant capacity (AC). Scanning electron micrographs (SEM) of flour showed that some starch granule was destroyed with germination. In the same way, pasting values decreased due to changes in the starch granule. FT-IR (Fourier Transform Infrared) spectra confirmed that germination leads to changes in the chemical structure. The XRD (X-ray diffractometer) analysis showed that patterns did not change with germination. In addition, raw and germinated corn flour were used in gluten-free cookie preparation. The cookie dough was evaluated for stickiness. The physical, textural, colour, TPC, and AC of cookie samples were determined. Gluten-free cookies prepared with germinated flour exhibited significantly higher AC due to increase in TPC. Germination could be used to enhance functional properties of corn.

KEYWORDS

antioxidant activity, cookie, corn, germination

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1. INTRODUCTION

The gluten-free term is generally known and is related to a certain group of the world population suffering from gluten-related diseases including celiac disease, which is the most well-known wheat allergies, wheat-dependent exercise-induced anaphylaxis (WDEIA), etc. (Khairuddin and Lasekan, 2021). However, it should be known that there are differences in these diseases, and knowing these differences is important for treatment (Pietzak and Kerner Jr., 2012). For example, people having wheat allergies should eliminate the wheat product from their diet due to the fact that these allergies are associated with the adverse immune response to different protein fractions of wheat (Scherf, 2019; Cabanillas, 2020). Lee et al. (2022) reported several gliadins and glutenins, especially ω-5 gliadin, as major allergen proteins causing WDEIA. On the other hand, celiac disease is known as gluten intolerance in food and abnormal intestinal T-cell responses to gluten proteins that are considered as triggering molecules in celiac disease (Sollid et al., 2012, 2020). In addition to gluten protein, celiac disease-relevant T-cell epitopes were also found in some glutenin proteins and non-gluten proteins, such as ATIs, seed storage globulins, or serpins (Juhász et al., 2020; Sollid et al., 2020). One percent of the world population is affected by celiac disease (Newberry et al., 2017; Khairuddin and Lasekan, 2021). Beside patients diagnosed the celiac disease, the rising trend on "gluten-free movement" has significantly increased in the general population due to an increase in number of cases and awareness of the population (Newberry et al., 2017).

Recently, researches about the development of different gluten-free food for celiac patients and enhancement in the structure of these products have increased. Corn is one of the safe cereals for celiac patients. Corn (*Zea mays* L. *everta*) belongs to the class in *Poaceae* and has widely cultivated all over the world. It has rich sources of high-quality proteins and carbohydrates; thus they play an important role in the daily diet (Serna-Saldivar and Carrillo, 2019).

In recent years, many researchers have focused on germination, which is an effective and inexpensive way to increase TPC and modify functional properties. There are several studies on germination in the literature. In most of them, the phytochemical compounds and nutritional value of grain have increased, thus, the grain became healthier for consumers. Some reserve ingredients can change during germination by enzymatic activity. The changes can lead to modification of the functional properties of grains. The utilisation of germinated flour for preparation of bakery foods has been suggested due to an increase in nutritional and phytochemical value and improvement in texture properties (Chauhan et al., 2015; Jan et al., 2016; Singh et al., 2017).

Soft wheat flour is used for cookie production traditionally. However, to produce gluten-free bakery products, corn, rice, and sorghum flours are widely used, and corn flour and starches allow for high quality of cookies, pasta products, and bread (Serna-Saldivar and Carrillo, 2019).

There are some studies present in the literature, where corn flour and corn flour blends were used in the gluten-free cookie formulation (Rai et al., 2014; Al Shehry, 2016; Korus et al., 2017; Obinna-Echem and Robinson, 2019; Umerah et al., 2020). However, there is little information on using of germinated corn flour for biscuit and cookie preparations (Adedeji et al., 2014; Agrahar-Murugkar et al., 2015). On the other hand, no information is available on using corn and germinated corn flours for cookie preparation without any flour fortification by comparing them and/or the effect of using both flours on antioxidants, nutritious, and physical properties of cookies. Therefore, in this study, we investigated the effect of germination on the functional, morphological, chemical structure, and pasting properties of corn. The effects of germination on the TPC, AC, physical and textural properties of cookies were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Corn kernels and all ingredients used in cookie-making were purchased from a local market. All chemicals were of analytical grade. Corn kernel samples from a single variety were used in this study.

2.2. Preparation of flour samples

The raw corn flour (RF) and germinated corn flour (GF) were prepared as described previously (Oskaybaş-Emlek et al., 2021). The germination was carried out for 4 days and two batches of samples were germinated under identical conditions.

2.3. Characterisation (RF and GF)

AOAC (1995) methods were followed to determine moisture (925.08), ash (923.03), lipid (945.16), protein (992.23) (N: 6.25), and total dietary fibre (TDF) (991.43) contents of flour samples.

Water activity meter (Novasina, Switzerland) was used to measure the water activity (a_w) of samples. The pH value of samples (10% (w/v) suspension) was determined with a pH meter (Hanna Instruments, Malaysia). Bulk density (BD) was determined using the method of Tatar et al. (2014). Oil absorption capacity (OAC), water absorption capacity (WAC), and water solubility index (WSI) were determined as per method reported by Oskaybaş-Emlek et al. (2021).

Rapid Visco Analyser (RVA 4500, Perten Instruments, Australia) and scanning electronic microscope LEO 440 SEM-EDX (Leica-Zeiss, DSM-960) were used to determine the pasting and morphological properties of samples (Oskaybaş-Emlek et al., 2021).

FT-IR (Fourier Transform Infrared Spectroscopy, Thermo Nicolet Avatar 370) was used to determine functional groups of samples with 4000–400 cm^{-1} scanning rate.

XRD (X-ray diffractometer) patterns of samples were obtained by X-Ray diffractometer (Bruker AXS D8, Germany). Relative crystallinity (RC) was calculated using the diffractometer's software.

2.4. Cookie preparation

Cookie samples were prepared as described by Oskaybaş-Emlek et al. (2021). The cookie formulation was as follows: 120 g of flour, 1.0 g of sodium bicarbonate, 20 g of skim milk powder, 40 g of ground sugar, 1 g of salt, 40 g of shortening, and 40 mL of water. Four cookies were produced per 120 g of flour source.

2.5. Dough stickiness

Dough stickiness was determined using texture analyser (TA.XT Plus texture analyser, UK) with an SMS/Chen-Hoseney dough stickiness rig (A/DSC) (Packkia-Doss et al., 2019).

2.6. Physical and textural properties of cookie samples

The diameter (D), thickness (T), and spread ratio (D/T) of samples were measured with a digital calliper. Texture analyser was used to measure cookie hardness (Oskaybaş-Emlek et al., 2021).

2.7. Colour properties (flour and cookie)

Colour properties of flour and cookie samples were determined by measuring L^* (brightness), a^* (red/green), and b^* (yellow/blue) using Konica Minolta CR 400 (Japan).

2.8. Total phenolic content (TPC) and antioxidant capacity (AC) (flour and cookie)

The TPC, DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2′-azinobis [3-ethyl-benzothiazoline-6-sulfonic acid]-diammonium salt) radical scavenging activity of samples were determined as reported previously (Oskaybaş-Emlek et al., 2021).

2.9. Statistical analysis

Three replicates were carried out for each proximate analysis, and four replicates were carried out for TPC and antioxidant analyses. For colour analysis, seven readings were done at different points on the surface of four cookie samples, and mean value was evaluated. Independent *t*-test ($P < 0.05$) was performed to compare the data of flour samples (RF & GF) and cookie samples (RFC & GFC) among themselves (SPSS Inc., Chicago, IL).

3. RESULTS AND DISCUSSION

3.1. Characteristics (RF and GF)

Characteristics of flour samples are presented in Table 1. The ash, TDF, and lipid contents of RF significantly ($P < 0.05$) decreased with germination. The vegetative growth led to the degradation of the outer bran layer in the cell, cell wall degraded as a result of vegetative growth, and the fat was hydrolysed by lipolytic enzymes with germination, thus, the ash, TDF, and lipid contents diminished (Singh et al., 2017). The protein content of RF decreased slightly with germination ($P > 0.05$).

The a_w and pH values of RF reduced with germination ($P < 0.05$). The drying following germination decreased the a_w. The a_w values indicated that the RF and GF were suitable for safe storage (Oskaybaş-Emlek et al., 2021). With germination, the complex molecules' hydrolysation into simpler acidic compounds led to reduction in the pH of samples (Dhillon et al., 2020).

Germination caused decrease in BD and WAC and increase in OAC and WSI of samples ($P < 0.05$). Hydrolytic enzymes (lipases, amylases, fibre-degrading, proteases, etc.) are activated with germination, thus, complex molecules begin to breakdown and convert into low molecular weight substances. Thus, particle heaviness and structure of starch and other molecules changed. These changes led to decrease in BD and WAC and an increase in the WSI of samples (Chauhan et al., 2015; Sharma et al., 2019). The released sugars after starch degradation formed crosslinks in the amorphous region of starch chains and also decreased the WAC (Singh et al., 2017). The change in surface accessibility of nonpolar amino acids and other nonpolar side chains with germination led to an increase in OAC (Sharma et al., 2019).

Table 1. Characteristics of corn flour samples

Parameters	Flour	
	RF	GF
Ash (%)[1]	3.72 ± 0.04*	2.22 ± 0.03*
Protein (%)[1]	12.86 ± 0.01	12.36 ± 0.40
Fat (%)[1]	10.65 ± 0.40*	9.50 ± 0.04*
TDF (%)[1]	19.35 ± 0.31*	17.23 ± 0.74*
pH	6.89 ± 0.03*	6.31 ± 0.06*
a_w	0.42 ± 0.01*	0.37 ± 0.00*
BD (g mL^{-1})	0.34 ± 0.01*	0.32 ± 0.00*
WAC (g g^{-1})	1.54 ± 0.03*	1.34 ± 0.01*
OAC (g g^{-1})	0.87 ± 0.00*	1.01 ± 0.01*
WSI (%)	12.69 ± 0.03*	13.36 ± 0.16*
L^*	83.77 ± 0.04*	82.40 ± 0.30*
a^*	−1.88 ± 0.01*	−1.78 ± 0.01*
b^*	23.27 ± 0.02*	23.80 ± 0.01*
PV (cP)	663.50 ± 1.50*	51.50 ± 0.50*
FV (cP)	1,326.00 ± 9.00*	68.00 ± 1.00*
SV (cP)	680.00 ± 10.00*	20.50 ± 0.50*
PT (°C)	80.70 ± 0.80	79.83 ± 0.02
TPC (mg GA/g)	1.60 ± 0.06*	2.52 ± 0.08*
DPPH (mmol Trolox/kg)	4.60 ± 0.29*	5.52 ± 0.17*
ABTS (mmol Trolox/kg)	6.91 ± 0.29*	11.49 ± 0.28*

[1]: Dry basis.
*: Means within each row are significantly different according to independent *t*-test ($P < 0.05$).

Germination led to decrease in L^* of RF ($P < 0.05$) due to increase in TPC (Jan et al., 2016). On the contrary, the a^* and b^* values of RF increased with germination ($P < 0.05$).

The GF showed lower peak (PV), final (FV), and setback viscosities (SV) than that of RF ($P < 0.05$). The decrease in PV may be related to starch degradation by amylolytic activities (Lee et al., 2019). In addition, the PV is related to the ability of starch to bind water (Li et al., 2019). The FV and SV are related to the capability of amylose molecules to aggregate (Chung et al., 2012). Therefore, the decrease in FV and SV may be due to degraded starch molecules, i.e., the decrease in amylose molecules caused lower aggregation of amylose and lower SV and FV values. The pasting temperature (PT) of RF slightly decreased with germination ($P > 0.05$).

The SEM image of RF (Fig. 1A) shows polygonal granule with a smooth surface (Xue et al., 2017), an intact starch granule embedded in protein matrix with a continuous structure. With germination, starch granule was deformed and protein matrix began to disappear (Fig. 1B). Figure 1 indicated that germination caused the degradation of starch and other molecules (Xu et al., 2017).

The FT-IR spectra of samples are shown in Fig. 2. The peaks at the range of 3,000–3,500 cm^{-1} could be attributed to O-H bond stretching of water molecules (Oskaybaş-Emlek et al., 2021).

The spectral bands at 2,922 cm^{-1} and 2,852 cm^{-1} corresponded to asymmetrical and symmetrical –CH stretches, respectively. The bands at 1,745 cm^{-1} related to the –C=O carbonyl bond from lipids (Xu et al., 2017), but the band was not observed in GF. A similar result was observed in germinated adlay by Xu et al. (2017). The peaks in the range of 1,641–1,650 cm^{-1}

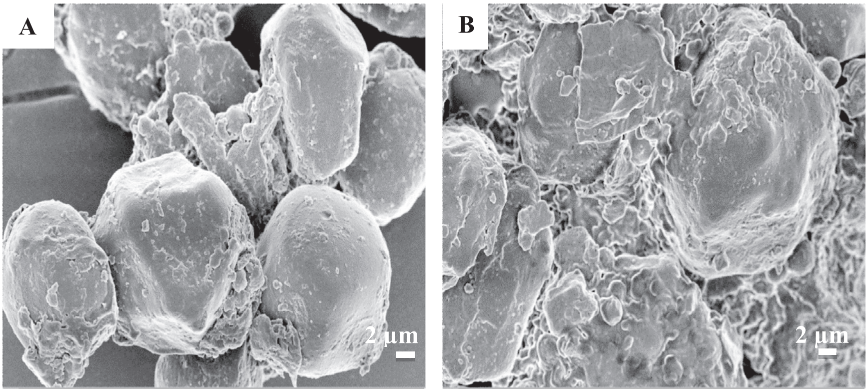

Fig. 1. SEM images of corn flour samples. a: RF, b: GF. magnification – 2.70 kX

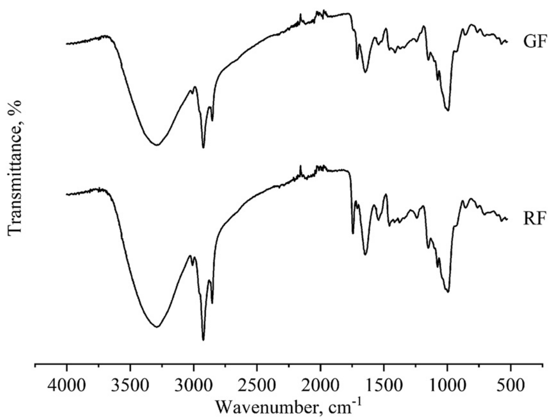

Fig. 2. FT-IR spectra of corn flour samples

were related to random coils structure. The peaks at 1,540 cm^{-1} indicated amide II structure (Ghumman et al., 2016). The bands at near 1,410 cm^{-1} and 1,455 cm^{-1} are attributed to symmetric and asymmetric CH_3 bending of the methyl groups. The peaks at the range of 1,200–900 cm^{-1} represent the polysaccharide region (Zhang et al., 2017).

Both flour samples exhibited A-type crystallinity characterised by the peaks at 15°, 17°, 18°, and 23° (Ma et al., 2020) (Fig. 3). Ma et al. (2020) also observed that corn starch belonged to A-type crystallinity with germination. On the other hand, germination led to decrease in RC due to starch degradation (Ma et al., 2020).

3.2. Dough stickiness

The utilisation of GF in cookie-making led to a decrease in dough stickiness ($P < 0.05$) due to a decrease in the WAC of flour. The increase in the WAC of flour caused an increase in dough stickiness (Agrahar-Murugkar et al., 2015; Patil et al., 2020). The addition of higher hydrophilic

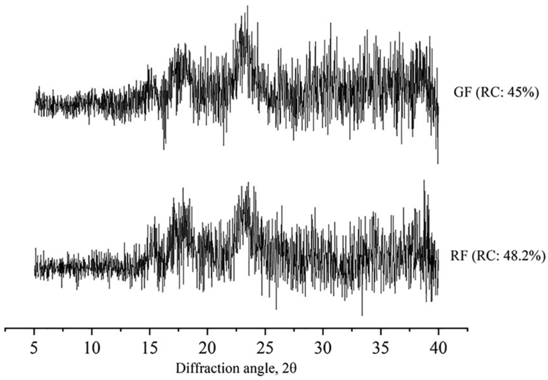

Fig. 3. XRD patterns of corn flour samples

flour to dough plays an important role in stickiness due to change in WAC (Farbo et al., 2020). Both dough samples can be accepted for good handling properties, as their stickiness values were lower than 0.98 N (Jin et al., 2020). There is a positive correlation between the PV of flour and dough stickiness (Jin et al., 2020). The PV of RF was higher than that of GF, and expectedly, the stickiness of dough prepared with RF was higher.

3.3. Characteristics (RFC and GFC)

The characteristics of cookies prepared with RF (RFC) and GF (GFC) are presented in Table 2. The D/T of RFC was slightly higher than that of GFC ($P > 0.05$). The GFC had a lower hardness value than RFC ($P < 0.05$). The degraded macromolecules with germination caused looser structural matrix formation and softer cookie texture (Jan et al., 2016).

Table 2. Characteristics of cookies

Parameters	Cookie	
	RFC	GFC
Stickiness (N)	0.42 ± 0.00*	0.37 ± 0.01*
Hardness (N)	23.90 ± 1.06*	19.35 ± 0.49*
D (mm)	60.00 ± 0.0*	56.00 ± 1.00*
T (mm)	8.50 ± 0.50	8.00 ± 0.00
D/T	7.08 ± 0.42	7.00 ± 0.13
L^*	70.05 ± 0.37*	60.98 ± 1.46*
a^*	0.43 ± 0.02*	4.71 ± 0.05*
b^*	33.85 ± 0.13*	31.65 ± 1.27*
TPC (mg GA/g)	1.91 ± 0.11*	2.79 ± 0.06*
DPPH (mmol Trolox/kg)	5.03 ± 0.16*	6.58 ± 0.12*
ABTS (mmol Trolox/kg)	7.64 ± 0.22*	13.72 ± 0.38*

*: Means within each row are significantly different according to independent *t*-test ($P < 0.05$).

The L^* values of RFC decreased with germination ($P < 0.05$). Increase in TPC of flour remarkably decreased the L^*, resulting in darker cookies (Jan et al., 2016). Another reason for the decrease in L^* of cookie can be due to brown melanoidin pigments formation with Maillard reaction during baking (Jan et al., 2016). The utilisation of GF in cookie-making led to an increase in a^* and decrease in b^* ($P < 0.05$). The GFC was darker, redder, and less yellow than RFC in terms of colour.

3.4. TPC and AC (flour and cookie samples)

The germination led to an increase in the TPC value of flour and cookie samples ($P < 0.05$) (Tables 1 and 2). With germination, the bound phenolics found in the cell walls are released by enzymatic activity, thus, TPC in corn increases (Gong et al., 2018). The TPC of RFC and GFC were higher than that of RF and GF, respectively. The increase could be attributed to Maillard reaction products having antioxidant properties formed during baking (Chauhan et al., 2015). The AC was evaluated in terms of DPPH and ABTS radical scavenging activity. The increase in TPC has resulted in improvement of AC. The DPPH and ABTS antioxidant activity of RF increased with germination ($P < 0.05$). Similarly, GFC had higher DPPH and ABTS antioxidant activity than RFC ($P < 0.05$). It may be attributed to the increase in hydrolytic enzymes with germination, thus, the availability of some bound phenolic compounds increased (Jan et al., 2016). Similar to TPC results, the AC of RF and GF was lower than RFC and GFC, respectively, due to the Maillard reaction, as stated above.

4. CONCLUSIONS

Recently, germination is gaining popularity around the world as it increases antioxidant activities of cereals. This study enhanced our understanding of the role that germination played on corn as well as the utilisation potential of germinated corn flour in cookie making. Germination affected the characteristics, chemical structure, morphological and pasting properties, total phenolic content and antioxidant capacity of corn. In addition, cookies were prepared using raw corn flour and germinated corn flour, and expectedly, the cookies prepared with germinated corn flour showed higher antioxidant capacity. Evaluating of all of data, germinated corn flour has great potential as functional food ingredient. The cookies prepared with raw or germinated corn flour could be an alternative for celiac patients.

ABBREVIATIONS

ABTS	2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt
AC	antioxidant capacity
BD	bulk density
D	diameter
D/T	spread ratio
DPPH	2,2-diphenyl-1-picrylhydrazyl
FT-IR	Fourier Transform Infrared Spectroscopy
FV	final viscosity

GF germinated corn flour
GFC cookies prepared with germinated corn flour
OAC oil absorption capacity
PT pasting temperature
PV peak viscosity
RC relative crystallinity
RF raw corn flour
RFC cookies prepared with raw corn flour
SEM scanning electron micrographs
SV setback viscosity
T thickness
TDF total dietary fibre
TPC total phenolic content
WAC water absorption capacity
WDEIA wheat-dependent, exercise-induced anaphylaxis
WSI water solubility index
XRD X-Ray diffractometer

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