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Production of hazelnut skin fibres and utilisation in a model bakery product

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RESEARCH ARTICLE

Abstract

Microfluidisation was used as a potential method for the production of fibrous structure from hazelnut skin (HS), and which was evaluated as an ingredient in cake as model bakery product. More specifically, effects of different amounts of microfluidised HS on rheological behaviour of cake batter and quality parameters (texture and colour) and storage characteristics of cake products were evaluated. Furthermore, performance of microfluidised HS was compared with conventionally milled HS and cacao powder for comparison purpose. The entangled structures of microfluidised HS resulted in much higher consistency index, yield stress and viscoelastic moduli values in batter samples than conventionally milled hazelnut skin. Highly shredded fibrous structure of microfluidised HS provided gluten-like strength and elasticity to cake samples resulting in higher springiness and firmness values. Darker crumb colour of the cake samples was caused by the homogenous distribution of fibrous microfluidised HS. Detailed staling analysis through X-ray and Fourier transform infrared measurements showed that microfluidised HS containing cake samples had lower retrogradation and staling tendency due to their lower starch content, and higher water holding ability. Findings of this study showed that microfluidised HS could be potentially utilised as an ingredient in bakery products.

Keywords: cake, fibre, hazelnut skin, microfluidisation, staling, texture

1. Introduction

Hazelnut is one of the popular nuts and it is ranked as second among tree nuts after the production of almond (Shahidi *et al.*, 2007). Turkey is among the world's largest hazelnuts producers with approximately three quarters of the global production (Anil, 2007). Hazelnut is therefore of great importance to Turkey's economy. While whole nut as raw or unroasted can be consumed, it can also be added to processed foods such as bakery and confectionery products. Hazelnut contains several parts including green leafy cover, shell and skin. Hazelnut skin (HS), 2.5% of the total hazelnut kernel, is obtained by a roasting process (Alasalvar *et al.*, 2009). Although HS is generally used as animal feed, some studies have shown that HS is a rich source of natural phenolic antioxidants and fibres. It has been reported that fibre content of hazelnut skin may reach up to 58.3% of the total composition at dry basis (Montella *et al.*, 2013; Schmitzer *et al.*, 2011; Yurttas *et al.*, 2000). Moreover, HS is rich in terms of phenolic compounds and six phenolic aglycones were identified in Turkish and American hazelnuts: gallic acid, sinapic acid, p-hydroxybenzoic acid, quercetin, caffeic acid and epicatechin (Montella *et al.*, 2013; Rio *et al.*, 2011; Yurttas *et al.*, 2000). Shahidi *et al.* (2007) indicated that HS had a higher total phenolic content than hazelnut kernel. Thus, HS phenolic extract might be used as a suitable source of natural dietetic flavonoids (Contini *et al.*, 2012).

As the interest in food products with high nutritional value increases, different additives are investigated and used in the food industry. In the search of new additives various parameters such as, water and oil holding capacity, phenolic content, colour, etc., are considered as important factors. Although, many researchers try to enrich bakery products with fibres, there are several drawbacks of fibre addition that must be considered. Decreasing volume, increasing hardness and prolonged baking times are some of the undesirable effect. There are number of papers conducted on the use of fibres from different sources in bakery products (Sreenath *et al.*, 1996). Figuerola *et al.* (2005) investigated apple pomace and citrus peel as a potential fibre source. In the study of Masoodi *et al.* (2002), cakes prepared with apple pomace wheat flour blends at 5, 10 and 15% were analysed. Anil (2007) used hazelnut testa as a source of dietary fibre in bread. Other than these studies, peanut hull, apple fibre, sugar beet fibre, wheat bran, oat bran, rice bran, whole grain rye, grape seed pomace, corn bran, carob fibre, soy hull, field pea hull, peanut hull and sunflower hull, psyllium husk fibre, and flaxseed are among the fibres used in cereal-based and bakery products (Acun and Gül, 2013; Babcock, 1987; Chen *et al.*, 1988; Collins *et al.*, 1982; Dreher and Padmanaban, 1983; Glitsøt and Bach Knudsen, 1999; Ozkaya, 1997; Park *et al.*, 1997; Pomeranz *et al.*, 1977; Riaz, 2001). In the study of Sudha *et al.* (2007), the acceptable rheological characteristics were obtained with the use of apple pomace as a source of dietary fibre and polyphenols in cakes.

Microfluidisation has been gaining popularity in food, pharmaceutical, biotechnology and cosmetics related research due to its size reduction ability under high pressure and shear rate. Furthermore, microfluidisation process can be used to obtain more uniform samples with very small dimensions. The instrument includes a reaction chamber in which the fluid is force to divide into two microstreams then the streams collide with each other at very high speeds (Cook and Lagace, 1987; Mert, 2012). Both high shear rate and extreme impact forces cause the formation of fine particle (McCrae, 1994; Mert, 2012). Valve homogenisation, colloid mill and dry milling techniques are commonly employed for size reduction purposes. However, conventional milling usually is not efficient enough to obtain ultrafine structures. Kim *et al.* (2013) and Zhu *et al.* (2010) reported that reduction of the wheat bran size below 40 μm is often considered difficult due to its soft structure and relatively low density. Microfluidisation has been shown to be an effective way to improve water holding capacity and free phenolic content of fibres (Mert *et al.*, 2014; Wang *et al.*, 2012). Although HS is known with its high phenolic content and dark colour like cacao, there is lack of value added products obtained from HS. Therefore, the purpose of this study was to improve functional properties of HS through microfluidisation and utilisation in a model bakery product. More specifically, the effects of microfluidised hazelnut skin (MF-HS) on quality parameters (texture, specific volume and colour) and storage characteristics of cake products were evaluated and compared with ball milled (BM-HS) and hammer milled hazelnut skin (HM-HS). This study also reports the rheological properties of cake batter samples containing different amounts of MF-HS, HM-HS, BM-HS, and cacao powder.

2. Material and methods

Materials

Cake (wheat) flour and cake shortening were obtained from ETI Food Industry Co. Inc. (Eskisehir, Turkey). Sugar, salt, baking powder, non-fat milk powder, egg white powder and cacao powder were supplied by Ulker Biscuit Industry Co. Inc. (Ankara, Turkey). HS was supplied from Sanset Food Tourism Industry Co. Inc. (Ordu, Turkey).

Hazelnut skin production

Three different types of HS samples were prepared to utilise in cake formulations. These samples were HM-HS, BM-HS, and MF-HS. HM-HS samples were simply went through a size reduction step using a hammer mill (Thomas-Wiley, Laboratory Mill, model 4; Arthur H. Thomas Company, Philadelphia, PA, USA). A sieve with aperture size 0.5 mm was used during this step. In case of BM-HS, size reduction was achieved using a ball mill (PM 100; Retsch, Haan, Germany) and the samples were sieved with 0.075 mm aperture size.

To produce MF-HS, a microfluidiser equipment (M-110Y; Microfluidics, Westwood, MA, USA) was used. Firstly raw HS samples were soaked in warm water for overnight for softening purpose. Then, the HS slurry was processed in the microfluidiser equipped with two Z-type interaction chambers having 200 and 100 μm openings. This process was repeated twice and the final slurry was freeze-dried (Alpha 2-4 LD plus; Martin Christ, Gefriertrocknungsanlagen GmBH, Osterode, Germany). All the samples were stored at -18 °C until further analysis.

Rheological measurements

The rheological measurements were carried out at 25 °C, using a TA rheometer (AR 2000ex; Rheometer, West Sussex, UK) with parallel plate geometry (40 mm diameter and 1 mm gap). The batter sample was placed between the plates and the edges were carefully trimmed with a spatula. The flow measurements were conducted where shear stress, τ was measured versus shear rate, γ that changed from 0.1 to 50 s^{-1}. In dynamic oscillatory experiments, first linear viscoelastic region of the samples was determined then all the experiments were conducted at 0.5% strain rate. The frequency sweep tests were done from 0.1 to 10 Hz. Finally, elastic (G') and viscous (G") moduli were obtained. Results were given as complex modulus (G*). All the rheological experiments were performed at least three times. Rheological measurements of the different concentrations of HM-HS, BM-HS, cacao powder, and MF-HS were also conducted using parallel plate geometry at 25 °C. Suspension were gently mixed using agitator for at least 3 h to ensure complete hydration and dispersion. All the rheological experiments were performed in triplicate and their averages were reported in the study.

Scanning electron microscopy analysis

Images of HM-HS, BM-HS, MF-HS and cacao samples were recorded with a scanning electron microscope (SEM; QUANTA 400F Field Emission SEM; FEI, Eindhoven, the Netherlands) at an accelerating voltage of 20 kV. Images were acquired at 100× and 250× magnification levels.

Analysis of cakes

Preparation of cake samples

Basic cake recipe on a 100 g flour basis consisted of 100 g water, 80 g sugar, 50 g shortening, 12 g milk powder, 8 g egg white powder, 6 g baking powder and 1.5 g salt. Cakes were prepared by partial replacement of flour with different percentages (0, 10, 15 and 20%) with HM-HS, BM-HS, MF-HS and cacao powder.

Cake batter was prepared using a mixer (5K45SS; Kitchen Aid, St. Joseph, MI, USA). At first, baking powder, shortening, and egg white powder were creamed for 1 min at low speed to get a fluffy cream and then milk powder, sugar, and salt were added. They were whipped together for 2.5 min at low speed, and finally flour, HS or cacao powder and water added simultaneously and mixed; first for 2 min at low speed, then for 1 min at medium speed and finally for 2 min at low speed. After complete mixing, 60 g of cake batter was poured into silicon moulds and baked in a conventional oven (Susler, Istanbul, Turkey) at 175 °C for average of 15 min. After baking, cakes were cooled at 25 °C for 1 h. Control cake samples were prepared without addition of any HS. Mainly because of its characteristic dark colour, cacao powder was also included in this study to have another set of reference samples in terms of colour and texture.

Colour analysis

The crumb colour of the cakes was measured using a Minolta CR-10 colour reader (Minolta, Osaka, Japan) using the CIE L*, a*, and b* colour scale. The a* value ranges from -100 (redness) to +100 (greenness), the b* value ranges from -100 (blueness) to +100 (yellowness) and L* value ranges from 0 (black) to 100 (white). Five measurements were made at different positions from the surface of the cake slides and the mean value was recorded.

Total colour change (ΔE) was calculated from the following equation:

$$\Delta E^* = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \quad (1)$$

Colour of control cake was selected as the reference point and its L*, a* and b* values were represented as L_0, a_0 and b_0 which were 95.44, 1.19 and 12.41, respectively.

Staling analysis

For the staling analysis, cakes were allowed to cool down for 1 h; then stored at 22±2 °C in vacuum packs for different storage times (0, 3, 6 and 9 days).

Texture analysis

Texture profiles of the cake samples were determined using a texture analyser (TA.XT Plus, Stable Micro Systems, Godalming, UK) equipped with a 30 kg load cell. The measurements were done in duplicate after 1 h cooling at 25 °C. Cake samples were sliced in 1 cm thickness and compressed with 1.5 cm diameter probe. The measurement settings were 1 mm/s pre-test speed, 1 mm/s test speed and 10 mm/s post-test speed with 60 s holding time at 25% strain rate. Four replicates from two different sets of baked samples were measured and averages of values were used for calculation of firmness and springiness.

X-ray diffraction analysis

X-ray diffraction analysis was done using CuKa (λ=1.54056) radiation with an Ultima IV X-ray diffractometer (Ultima, Rigaku, Japan) at 40 kV and 40 mA. The scanning region of the diffraction angle (2θ) was 5-45° with the scanning speed of 2°/min. The analysis was performed using PeakFit version 4.12 software (SeaSolve Software, Richmond, CA, USA). After the samples were freeze-dried and milled, they were mounted with 0.5-1 mm thickness on a place of 2×2 cm^2 glass holder. Crystalline peaks were analysed as pseudo-Voight-form and the amorphous ones as Gaussian-form peaks. The crystallinity levels in the samples were determined by the separation and integration of the areas under the crystalline and amorphous X-ray diffraction peaks. The quantification of relative crystallinity was determined using total mass crystallinity grade (TC) in which the area of the crystalline fraction (dots) is divided by the crystalline fraction (solid line) plus the amorphous fraction (dash line), based on the method described by Ribotta *et al.* (2004) and Demirkesen *et al.* (2014):

$$TC = \frac{I_c}{I_c + I_a} \quad (2)$$

Where, I_c is the integrated intensity of crystalline phase, and I_a is the integrated intensity of the amorphous phase. The measurements were done in duplicate.

Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FT-IR) experiments were performed on a IR Affinity-1 Spectrometer (Shimadzu Corporation, Kyoto, Japan). The analysis was done in the middle-IR region, 600-4,000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans. Freeze-dried cake samples were put into the crystal surface by providing contact of attenuated total reflexion crystal (Shimadzu Corporation, Kyoto, Japan) with the sample. Two replications were done. The analysis was performed using PeakFit version 4.12 software. The measurements were done in duplicate.

Statistical analysis

Analysis of variance (ANOVA) was performed to determine whether there were significant differences between cake types (control, cacao powder, HM-HS, BM-HS and MF-HS), flour replacement levels (10, 15 and 20%) and storage times (0, 3, 6 and 9 days) ($P \leq 0.05$). If significant difference was obtained, means were compared by the Tukey Single Range test ($P \leq 0.05$) using MINITAB (version 16) software (Minitab Inc., State College, PA, USA).

3. Results and discussion

One of the main objectives of this study was to characterise the effect of various size reduction processes on HS. SEM images of HM-HS, BM-HS and MF-HS are given in Figure 1. Commercial cacao powder was also included for comparison purpose. As indicated in Figure 1A, cacao powder composed of small particles with homogenous size distribution. In case of HM-HS, relatively larger sieving size was used so it contained relatively larger particles (Figure 1B). Ball milling is known to be an effective size reduction technique. Therefore, the picture shown in Figure 1C had smaller particles similar to cacao powder. However, the picture depicted in Figure 1D had completely different structure. Overall appearance of MF-HS shown in Figure 2 indicates that microfluidisation of HS produced a fibrous and flaky structure. Rather than the small particles seen in BM-HS, MF-HS structure was made of extremely thin (1-2 μm) flakes and fibres. Microfluidisation is a high shear rate and pressure process, thus the operation particles with higher aspect ratio are obtained compared to ball milling and hammer milling. It is known that fibres tend to intertwine and form a network structure when the quantity is sufficiently high. These entangled structures contribute significantly to overall rheological properties of batters. Another important consequence of the structure shown in Figure 2 is its relatively higher water holding ability. As shown in Figure 2, MF samples were composed of extremely thin structures with very large surface area that might have higher number of hydrophilic moieties interact. This situation is clearly demonstrated in Figure 3 where suspensions of 20% (w/w) cacao powder, HM-HS, BM-HS, and MF-HS are shown. Cacao Powder, HM-HS, and BM-HS samples had liquid like structure whereas MF-HS sample had completely different semi-solid structure, which can maintain its shape even under modest deformation. This distinctive structure was probably caused by the fibrous formation shown in Figure 2. Visual differentiation shown in Figure 3 was also quantified with flow data given in Figure 4. As depicted in Figure 4, MF-HS had much higher apparent viscosity values than the other samples, for example example apparent viscosity of MF-HS was 3 orders of magnitude higher than that of HM-HS.

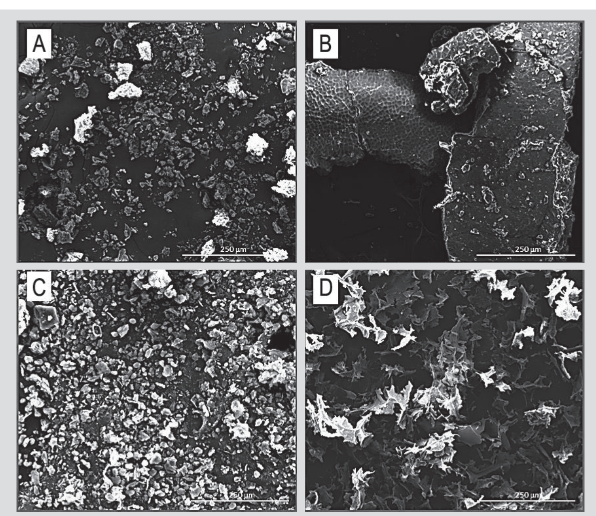

Figure 1. Scanning electron microscope images at 500× magnification. (A) cacao powder. (B) hammer milled hazelnut skin. (C) ball milled hazelnut skin. (D) microfluidised hazelnut skin.

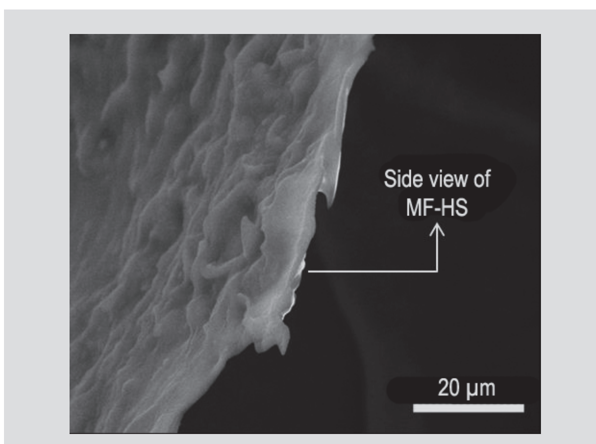

Figure 2. Scanning electron microscope image of microfluidised hazelnut skin (MF-HS) at 5,000× magnification.

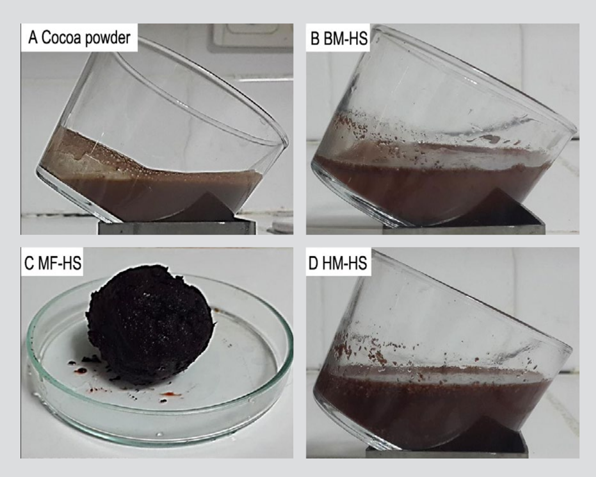

Figure 3. Suspensions of (A) cacao powder, (B) hammer milled hazelnut skin, (C) ball milled hazelnut skin and (C) microfluidised hazelnut skin at 20% (w/w) concentration.

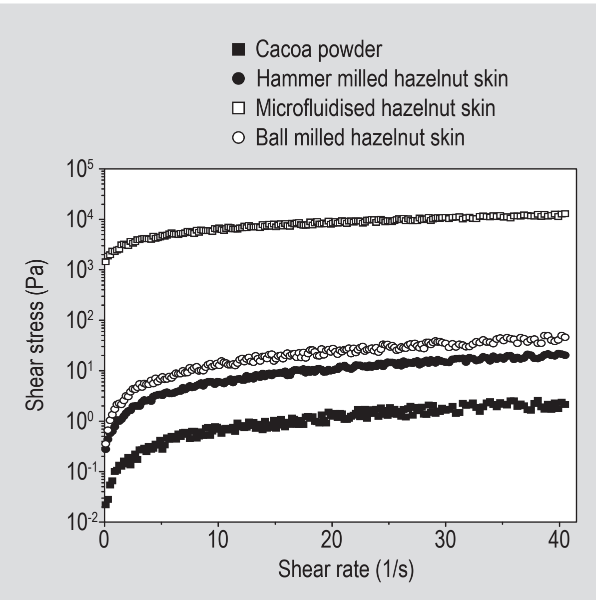

Figure 4. Flow properties of cacao powder, hammer milled hazelnut skin, ball milled hazelnut skin and microfluidised hazelnut skin at 20% (w/w) concentration.

Flow data of cake batter samples containing HM-HS, BM-HS, MF-HS and cacao powder are given in Table 1. The shear stress (τ) versus shear rate (γ) data were fitted well to the Herschel-Bulkley model (Equation 3) for all batter formulations at 25 °C:

$$\tau = \tau_o + K(\dot{\gamma})^n \quad (3)$$

Where, τ is the shear stress (Pa), τ_o is the yield stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($Pa \times s^n$) and n is the flow behaviour index.

Table 1. Herschel-Bulkley parameters of control batter and batter samples prepared with the replacement of flour with hazelnut skin (HS) samples and cacao powder at different percentages (10, 15 and 20%).

Formulation	τ_o (Pa)	K ($Pa.s^n$)	n	r^2
Control	52±1.4	24±2.1	0.53±0.06	0.99
10% cacao	71±3.2	27±1.4	0.56±0.04	0.98
15% cacao	81±4.8	31±1.7	0.51±0.06	0.98
20% cacao	96±4.1	51±2.3	0.51±0.07	0.97
10% HM-HS	87±3.8	34±1.6	0.61±0.03	0.98
15% HM-HS	94±5.2	51±2.1	0.55±0.08	0.98
20% HM-HS	111±7.1	89±3.3	0.55±0.04	0.98
10% BM-HS	98±8.8	51±3.1	0.53±0.05	0.97
15% BM-HS	141±10.1	71±4.4	0.53±0.05	0.98
20% BM-HS	184±10.8	101±5.2	0.53±0.06	0.97
10% MF-HS	214±15.4	74±3.2	0.49±0.04	0.99
15% MF-HS	291±17.3	98±5.1	0.49±0.02	0.98
20% MF-HS	446±22.1	168±9.6	0.46±0.09	0.99

BM = ball milled; HM = hammer milled; MF = microfluidised; τ_o is the yield stress; K is the consistency index; n is the flow behaviour index.

Table 1 shows the Herschel-Bulkley model parameters for the batter samples. Flow behaviour indexes of all batters were found to be lower than 1 that showed their shear thinning behaviour (pseudoplastic). Since the interactions between components were broken down under the action of shear, viscosity of batters decreased with the increasing shear stress (Demirkesen *et al.*, 2010a). The flow behaviour index values were between 0.46 and 0.61. Batter samples with the highest level of MF-HS had the lowest n value as 0.46. Switzer and Klingenberg (2003) stated that the elevated fibre concentration in a suspension increases yield stress due to the network formation between fibres. On the other hand, same study also demonstrated that the flow behaviour index (degree of shear thinning) decreased with increasing fibre concentrations.

As indicated in Table 1, among all batter samples, the lowest consistency index value was obtained from the control sample (prepared without flour replacement). Shear stress versus shear rate data indicated that flour replacement level influenced the rheological properties of the batter samples. Consistency value of batter samples is a significant parameter because there is a correlation between consistency and the air retaining capacity (Gómez *et al.*, 2010). Higher flour replacement levels led to elevated consistency index values of the batter samples. Yield stress is one of the significant rheological parameters to predict product's performance. It is defined as the stress level to initiate flow and it is related to internal interactions in a material (Mert *et al.*, 2014; Tabilo-Munizaga and Barbosa-Canovas, 2005). As depicted in Table 1, in accordance with the consistency index values of batter samples, the lowest yield stress values were observed in control batter samples. Similar to consistency index values, the increases in the flour replacement levels increased the yield stress values, which showed necessity of higher force to deform batter samples.

When the effects of flour replacement with HS samples or with cacao powder on the rheological properties of batter samples were examined, the observed consistency index and yield stress values were in the following increasing order: cacao powder, HM-HS, BM-HS and MF-HS. If the samples containing cacao powder and HS are compared, HS containing samples had higher consistency index and yield stress values. This may have been caused by the relatively higher fibre content of HS (around 60% by weight) compared to cacao powder (around 30% by weight) (Baba *et al.*, 2001; Montella *et al.*, 2013). Entanglement of fibre generates a resistance to flow and leads to increases in apparent viscosity and yield stress values. Because of higher water holding capacity of fibres, the available water in the mixture decreases resulting in diminished plasticising effect of water. Therefore, higher consistency index and yield stress values were obtained from the batter samples containing HS. The differences in flow properties of HS containing samples (HM-HS, BM-HS and MF-HS) can be explained with the differences in size and morphology of the added HS. As shown in Figure 1, HM-HS had bigger particles than BM-HS and MF-HS had completely different morphology with its fibrous and flaky structure. Studies with wheat bran and other fibre sources showed that decreasing particle size provides efficient mixing with increased consistency (Masoodi *et al.*, 2002; Zhang and Moore, 1997).

As shown in Table 1, among the cake batters containing HS samples, MF-HS had the most noticeable effect on yield stress and consistency index values of batter samples. The yield stress and consistency index values of MF-HS containing samples even at the lowest concentration (10%) were noticeably higher than that of the highest concentration (20%) BM-HS containing samples. This may be related to higher water holding capacity of MF-HS samples due to their more branched structure and larger surface area as shown in Figure 3. Microfluidisation process disintegrates flaky structures of bran in fibrous arrangements, causing a significantly higher surface area as shown in Figure 1 and 2.

Complex moduli ($G^* = \sqrt{(G')^2 + (G'')^2}$) values of cake batter samples are shown in Figure 5, 6 and 7. In all samples, both G' and G" values increased with angular frequency. Although it was not reported here separately, G' was found to be higher than G" indicating a solid like behaviour of the batter samples. G* modulus values of cake batter samples increased as flour was replaced with cacao powder and HS samples prepared by using different methods. In accordance with their viscosity values, further increases in complex modulus values of batter samples were obtained with elevated flour replacement levels. As indicated in Figure 5, 6 and 7, at the same flour replacement level, the effect of replacement of flour with MF-HS led to much higher increases in G* modulus values. The intertwining fibrous structure and higher water holding capacity of MF led to increases in G* modulus values of cake batters.

Effect of HS addition on baked cake samples were also evaluated. The impact of flour replacement level (10, 15 and 20%) and cake type (control, cacao powder, HM-HS, BM-HS and MF-HS) on firmness values of fresh cakes (day 0) are depicted in Table 2. ANOVA results showed that firmness values were significantly affected by cake types and flour replacement levels ($P \leq 0.05$). Increasing replacement percentages of flour with HM-HS, BM-HS, MF-HS and cacao powder led to higher firmness values in all cake samples. When HM, BM, and MF treatments were compared, the highest firmness values were obtained from MF-HS containing cakes, which were followed by BM-HS, HM-HS and cacao powder.

The differences between firmness values of HS and cacao containing samples might be explained by higher fibre content of HS. Among the HS containing samples, MF treatment resulted in significantly higher firmness values. For example, 10% MF-HS containing samples had higher firmness values than 20% BM-HS containing samples. This

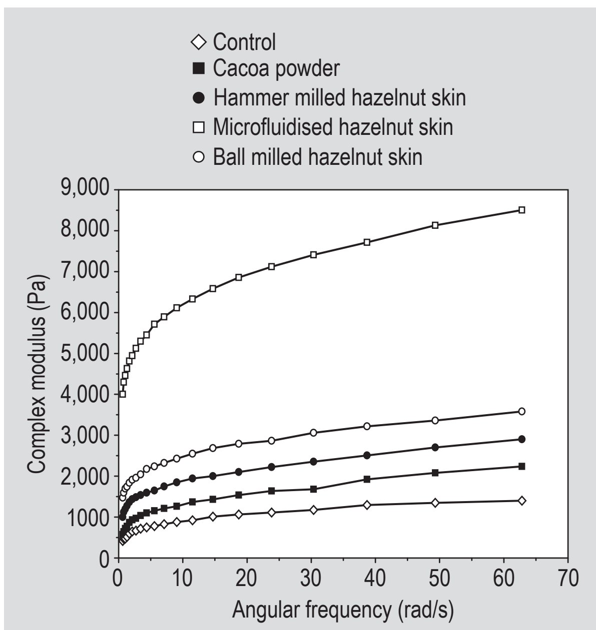

Figure 5. Complex modulus values of control batter and batter samples prepared with replacement of flour with hazelnut skin samples and cacao powder at 10%.

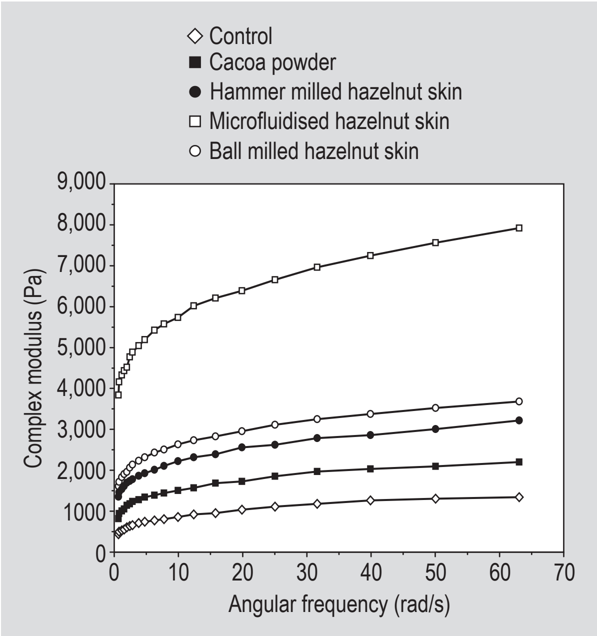

Figure 6. Complex modulus values of control batter and batter samples prepared with replacement of flour with hazelnut skin samples and cacao powder at 15%.

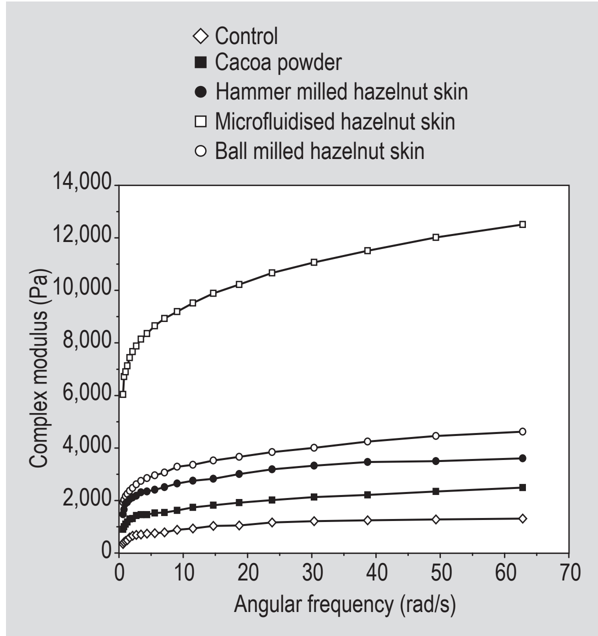

Figure 7. Complex modulus values of control batter and batter samples prepared with replacement of flour with hazelnut skin samples and cacao powder at 20%.

Table 2. Firmness and springiness values of control cake samples and cake samples prepared with the replacement of flour with hazelnut skin (HS) samples and cacao powder at different percentages and stored at different days (0, 3 and 6 days).

	Firmness (g)			Springiness (%)		
	Day 0	Day 3	Day 6	Day 0	Day 3	Day 6
Control	85.19±3.5	168.38±5.7	268.23±2.1	48.11±0.2	40.40±1.0	37.38±1.4
10% cacao	88.97±1.5	193.76±9.9	273.13±8.0	46.10±0.2	42.87±0.5	39.82±0.8
15% cacao	93.05±4.0	202.21±6.2	266.63±10.1	45.59±0.1	43.69±0.7	41.85±0.4
20% cacao	105.82±1.2	225.37±14.2	289.97±5.2	44.90±1.1	43.28±1.5	42.02±0.0
10% HM-HS	104.05±1.5	175.83±3.3	222.83±3.6	48.00±0.5	43.93±0.0	42.27±1.6
15% HM-HS	112.66±2.8	222.28±21.2	274.74±8.2	47.93±0.7	47.66±0.2	43.24±1.7
20% HM-HS	133.98±6.1	250.33±16.5	352.40±30.5	47.69±0.4	48.77±0.4	45.95±0.7
10% BM-HS	125.61±3.5	228.49±21.9	313.56±6.4	47.77±0.2	45.30±0.3	41.93±0.7
15% BM-HS	132.25±9.6	262.91±17.9	398.21±15.6	46.11±1.1	45.61±0.1	45.94±0.5
20% BM-HS	175.61±3.0	357.25±4.6	444.39±4.8	46.10±0.3	47.70±0.5	45.34±0.7
10% MF-HS	246.04±9.0	447.45±10.1	584.65±17.8	57.24±1.1	57.03±1.0	52.95±1.2
15% MF-HS	271.89±5.4	460.35±20.3	652.96±4.0	56.60±0.7	59.05±0.9	56.35±0.4
20% MF-HS	467.35±8.1	765.17±11.6	1,149.17±19.1	50.88±1.3	56.63±0.9	52.72±0.5
35% MF-HS	205.18±16.0	305.99±20.1	397.64±26.4	56.24±1.5	58.48±0.2	56.00±0.6
50% MF-HS	160.00±11.5	191.81±4.6	238.15±10.1	56.13±1.2	57.42±0.2	60.82±0.3
65% MF-HS	92.66±4.8	172.30±7.1	217.97±19.9	55.05±1.2	59.37±0.3	60.19±0.2

BM = ball milled; HM = hammer milled; MF = microfluidised.

was most probably caused by the structural differences shown in Figure 1 and 3. MF treated samples could form a network due to their highly shredded fibrous structure which might add gluten-like strength and elasticity to cake samples. MF treatment was also shown to improve the water holding ability of HS (Figure 3). Therefore, in MF-HS containing cake samples, higher firmness values were observed. Fibre content has a critical role in quality of baked products. Previous studies reported that the presence of fibre might enhance the quality parameters of baked products unless it exceeds certain level (Demirkesen *et al.*, 2010b; Lebesi and Tzia, 2011). Sudha *et al.* (2007) showed that the hardness of the cake samples prepared with apple pomace increased with the increasing percentage of fibre. Rupasinghe *et al.* (2009) obtained similar results for fruit fibres. The reason was attributed to the possible impeding effect of fibre in intermolecular interaction (Collar *et al.*, 2007). Fibre may restrict available water for gluten development and disrupt starch-gluten matrix. This impedes expansion ability of dough (Ktenioudaki and Gallagher, 2012). Thus, a compact structure can be obtained in the final product when higher level of fibre is used (Demirkesen *et al.*, 2010b). The impacts of the percentages and cake types on springiness values of fresh cakes were also shown in Table 3. ANOVA results showed that springiness values were significantly affected by cake types and flour replacement levels ($P \leq 0.05$). Among all cake samples, the highest springiness values were obtained from MF-HS containing cake samples indicating their more elastic structure.

In order to eliminate undesirable firmness increases due to MF-HS (fibre) addition, flour content was further reduced in this study. As shown in Table 2, 35, 50 and 65% replacement of flour with MF-HS were also tested. When replacement level exceeded 35%, firmness values decreased. For example, 65% replacement of flour resulted in similar firmness values as control sample. Same replacement levels were also tested for HM-HS and BM-HS, but these samples had inferior quality features with unacceptable large pores and collapsing soft structures. Therefore, texture analysis studies could not be conducted for HM-HS and BM-HS. Figure 8 depicts the pictures of the cake samples prepared with the replacement of flour with HS samples and cacao at different percentages. Cake samples prepared with HM-HS had distinctly different appearance than the other cake samples. Generally, these samples had large pores with relatively weaker crumb structure. Heterogeneous distribution of HM-HS particles was also noticed. When the HS was further milled into finer particles using ball milling, certain improvements in the cake structures were observed. When BM-HS was added into batter formulations, more homogenous crumb structure, similar to the cacao powder added samples, was obtained. This can be regarded as an expected result because both cacao powder and BM-HS had relatively finer particles (d(0.5) = 43.4 μm for BM-HS, and d(0.5) = 18.3 μm for cacao powder). In terms of pore structure and volume, MF-HS containing samples had similar features to cacao and BM-HS containing cake samples. However, colour and texture (Table 2 and 3 and Figure 8) of MF-HS containing samples were significantly different from those of the other samples. The cake samples prepared with higher flour replacement levels (35, 50 and 65%) had relatively homogenous pore distribution. It should be noted that when 65% of the flour, originally present in control cake samples, was replaced with MF-HS, the resulting sample had firmness and pore distribution similar to the control sample.

Table 3. Colour values (L*, a* and b*) of cake samples prepared with the replacement of flour with hazelnut skin (HS) samples and cacao at different percentages (10, 15 and 20%).

Formulation	L*	a*	b*
Control	95.44±0.7	1.19±0.3	12.41±2.3
10% cacao	53.58±1.3	18.54±4.3	17.20±4.2
15% cacao	44.90±3.5	16.06±2.1	15.04±1.4
20% cacao	37.45±1.1	15.69±3.0	13.64±1.0
10% HM- HS	66.63±1.3	25.94±2.3	21.61±4.3
15% HM- HS	60.45±1.3	27.55±2.2	23.50±2.3
20% HM- HS	56.36±3.9	30.48±3.8	23.87±2.8
10% BM-HS	53.68±1.8	34.05±7.7	17.83±6.9
15% BM-HS	38.22±4.2	39.61±3.7	16.32±4.2
20% BM-HS	29.89±2.3	41.53±2.8	18.71±3.9
10% MF-HS	22.62±2.1	14.41±2.3	7.10±2.0
15% MF-HS	15.08±1.1	10.79±3.6	5.51±2.8
20% MF-HS	11.77±2.1	7.75±2.0	4.31±1.5

BM = ball milled; HM = hammer milled; MF = microfluidised.

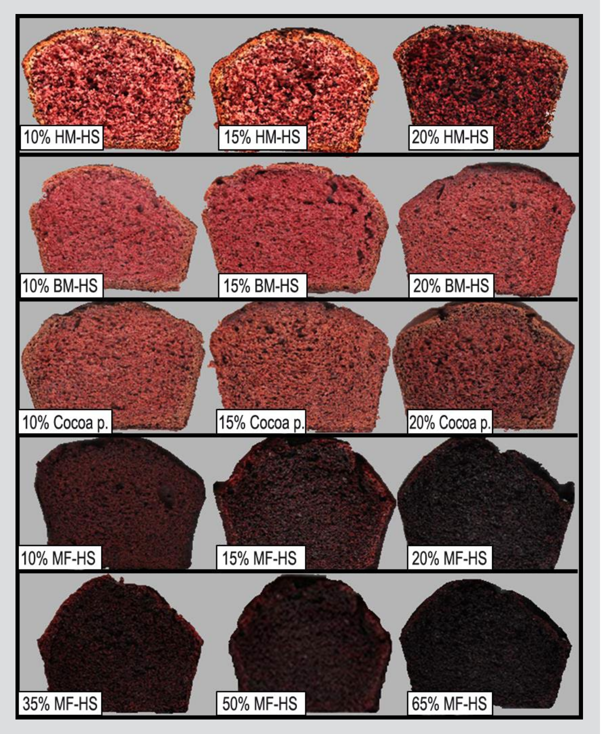

Figure 8. Pictures of different cake samples prepared with the replacement of flour with hazelnut skin samples and cacao powder at different percentages (10, 15 and 20%). Bottom row involves higher replacement percentages (35, 50 and 65%) of microfluidised hazelnut skin (MF-HS).

As presented in Figure 8, milling method of HS led to significantly different colour formation in cake samples. Results of the crumb colour measurements of cake samples prepared with the replacement of flour with cacao powder, HM-HS, BM-HS and MF-HS are given in Table 3. Both cake type (control, cacao powder, HM-HS, BM-HS and MF-HS) and the flour replacement levels (10, 15 and 20%) significantly affected the crumb colour of cake samples ($P \leq 0.05$). Replacement of flour with cacao powder, HM-HS, BM-HS and MF-HS fibre led to lower L* values indicating increased darkness of the crumb colour. Further decreases in the L* values of the cake crumbs were observed at higher flour replacement levels. However, no variations were observed for b* values of the cake samples. It has been stated that the increases in temperature are not high enough to promote browning reactions (Gómez *et al.*, 2010) and thus the decreases in in the L* of cake crumbs with the replacement of flour with cacao powder, BM-HS, BM-HS and MF-HS fibre might be related to the naturally darker colour of raw materials (Mert *et al.*, 2014). Similarly the differences in the a* value of cake samples can be explained by the natural colour of raw materials shown in Figure 8.

When the effects of HM, BM, and MF treatments on colour parameters of the cake samples were compared, the lowest L* values were obtained from MF-HS containing cakes, which were followed by BM-HS and HM-HS. The variations colour parameters of HS containing samples might be explained by the structural differences of samples such as size and morphology differences. HM-HS resulted in more heterogeneous distribution of particles and thus among HS containing samples the highest L* were obtained from HM-HS containing cake samples. On the other hand, the lowest L* values of MF-HS containing cake samples can be explained by the more homogenous distribution of fibrous MF-HS (Figure 1). Furthermore, wet milling process through microfluidisation might have caused release of the phenolic compounds, which were initially trapped within the cellular structure.

Staling which refers to all physical, chemical and sensorial changes occurring in baked products during storage that affects consumer acceptance. It involves retrogradation of amylopectin, interactions of polymers within the amorphous region, moisture loss, and distribution of water content between the amorphous and crystalline zone. Therefore, staling is regarded as an extremely complex process to describe (Gray and BeMiller, 2003). Effects of flour replacement level, cake type and storage time on firmness values of cake samples were found to be significantly different ($P \leq 0.05$). As indicated in Table 2, the firmness values of all cake samples increased during storage. For the fresh samples, the highest firmness value was obtained from MF-HS containing cake sample, and it was followed by BM-HS, HM-HS and cacao powder containing cake samples. However, at the end of 6 day storage period, percentage increases in the firmness values of MF-HS containing cake samples was less than all other cake samples. According to ANOVA results, it was found that springiness values were dependent on flour replacement levels, cake types and storage times. Decrease in springiness value can be considered as an indicator of staling. When the springiness values were compared for cake samples, MF-HS containing ones displayed relatively constant springiness values through the storage period.

X-ray diffraction analysis has also been used to examine the retrogradation behaviour of cake samples. For the X-ray analysis, cake samples prepared by replacement of 10% of flour with cacao powder, BM-HS, HM-HS and MF-HS were used. In this study, the crystallinity levels in the samples were determined by the separation and integration of the areas under the crystalline and amorphous X-ray diffraction peaks and the quantification of relative crystallinity was determined using total mass crystallinity grade in which the area of the crystalline fraction is divided by the crystalline fraction (solid line) plus the amorphous fraction (dash line) as indicated in Figure 9. The X-ray diffraction diagrams of fresh cake samples showed a peak at around 2θ of 20°,

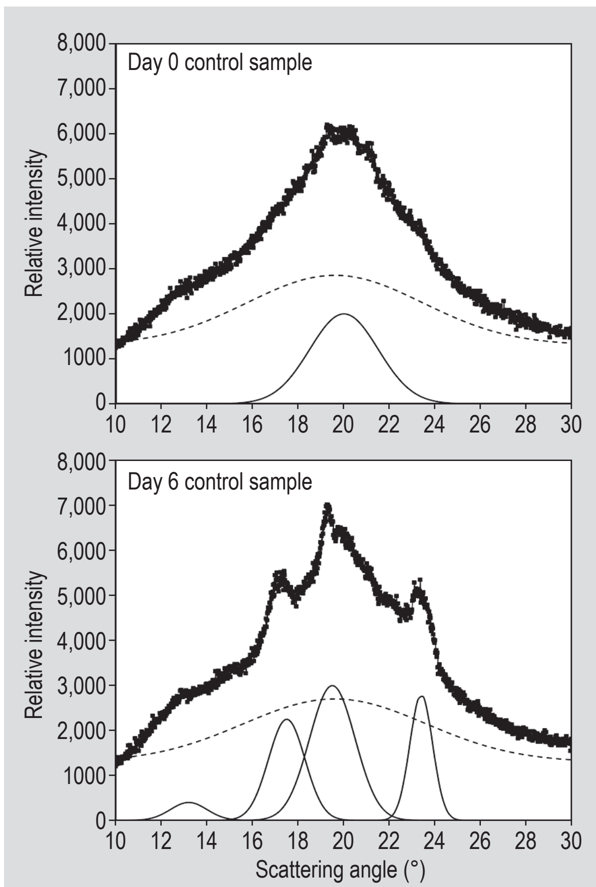

Figure 9. Typical X-ray diffraction peaks of control cake samples (dots = experimental data; solid line = fitted crystalline fraction; dashed line = fitted amorphous fraction).

corresponding to a typical V-type structure formed by the helical clathrates complex between amylose and fatty acids in cake samples (Demirkesen *et al.*, 2014). Starch is mostly in the amorphous form in freshly baked sample but it slowly recrystallises during aging (Karim *et al.*, 2000). During the staling period, the peaks present in fresh cakes usually observed at around 2θ of 20°, remained unchanged. In addition, all aged cake samples had peaks at 17° and 24° indicating B-type structure. They appear due to the crystallisation of the amorphous starch melt, mainly of the amylopectin fraction and their intensities increased for longer storage times. This result was in agreement with previously reported studies (Demirkesen *et al.*, 2014; Osella *et al.*, 2005; Ribotta *et al.*, 2004).

Figure 10 shows the total mass crystallinity grades of the cake samples. Starch retrogradation results in formation of more organised starch structure (crystallites) by the interchain association of the amylose and amylopectin fractions. As shown in Figure 10, higher crystallinity values were observed for cake samples stored for longer periods. The highest crystallinity grade values were obtained in control cake samples. This might be due to the higher total starch content, hence the amylose content of control cake samples. The replacement of flour by HS or by cacao powder led to decreases in available starch content for crystallisation and hence decreases in crystallinity values of cake samples (Demirkesen *et al.*, 2014). Among the cake samples containing HS, the lowest crystallinity values were obtained from MF-HS containing cake samples. As discussed before, microfluidisation of HS resulted in formation of a fibrous and flaky structure. These extremely thin (1-2 μm) flakes and fibres (Figure 2) might interact with more starch and prevent starch-starch interactions, thereby decreasing availability of organised starch for crystallisation. Furthermore, relatively higher water holding ability of MF-HS samples (Figure 3) might also prevent water loss during storage and reduce the effective water content associated to starch, which is required for amylopectin recrystallisation. Therefore, among cake samples containing HS, MF-HS containing cakes had the lowest retrogradation and staling tendency due to lower starch content, higher water holding ability and highly shredded fibrous structure.

The retrogradation behaviour of cake samples was also investigated using FT-IR spectroscopy. Peaks at 1,040 cm^{-1} is usually considered to be related to crystalline regions of starch and the peak at 1,150 cm^{-1} is often used as an 'internal correction standard peak' to normalise uncontrollable factors (Demirkesen *et al.*, 2014). Thus, in this study, the integral area ratios of peaks around 1,040 and 1,150 cm^{-1}, associated to the progressive ordering of the amylopectin polymer present in cake samples, were used to monitor starch retrogradation (Demirkesen *et al.*, 2014; Smits *et al.*, 1998; Van Soest *et al.*, 1994, 1995). The integral area ratios of peaks around 1,040 (A_1) and 1,150 cm^{-1} (A_2) are

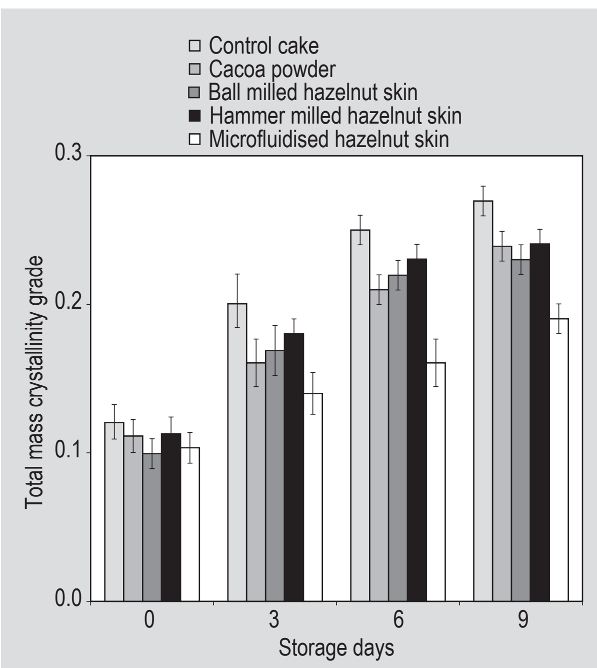

Figure 10. Total mass crystallinity grades of different cake samples prepared with the replacement of 10% flour with hazelnut skin samples and cacao powder and control cake samples at different storage times (0, 3, 6 and 9 days).

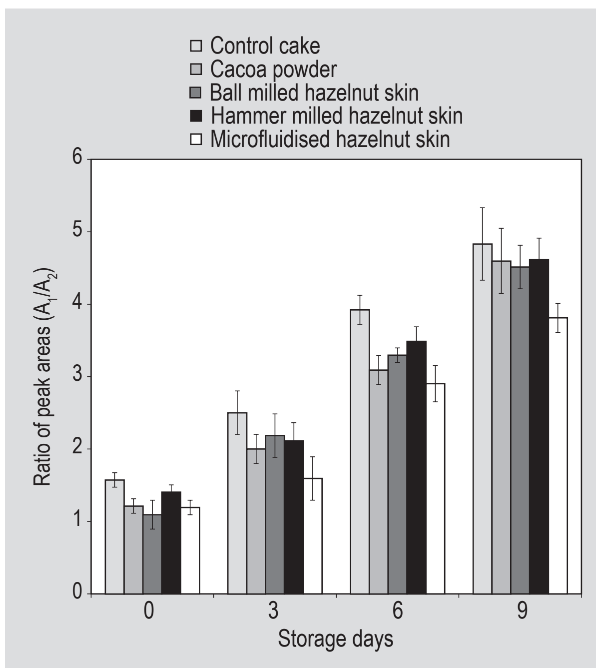

Figure 11. The integral peak area ratios of peaks around 1,040 cm^{-1} (A_1) and 1,150 cm^{-1} (A_2) of different cake samples prepared with replacement of 10% flour with hazelnut skin samples and cacao powder and control cake samples at different storage times (0, 3, 6 and 9 days).

given in Figure 11. A_1/A_2 increased with increasing storage time in all samples during storage. Among all cake samples, control cake samples had the highest A_1/A_2 value and replacement of flour by HS or by cacao powder reduced A_1/A_2 values of the cake samples. The lowest A_1/A_2 value was observed for MF-HS containing cake samples and this result revealed that starch retrogradation of cakes was retarded by replacement of flour with MF-HS. The findings from both FT-IR and X-ray analysis were found to be in a good agreement.

4. Conclusions

Although HS is rich and inexpensive source of natural antioxidant, it is often considered as waste product by hazelnut industry. This study evaluates various milling techniques with special emphasis on microfluidisation to produce a functional food ingredient from HS. SEM images revealed that microfluidisation produced highly branched fibrous structure from HS with improved water binding capacity. When it is evaluated as an ingredient in cake, it produced cake products with higher firmness and springiness and longer shelf life than those incorporated with conventionally milled HS. Colour of the cake samples was also changed significantly with type of the milling, MF-HS containing samples had darker colour which may be desirable for certain bakery products. Detailed stalling analysis was also conducted; X-ray patterns and FT-IR chromatograms showed that MF-HS delayed stalling through holding water and reducing starch retrogradation.

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