

# Hydroxymethylfurfural content and physicochemical properties of the caramel samples enriched with different dietary fibres

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# **RESEARCH ARTICLE**

### Abstract

Caramel is widely used in foods such as ice cream, biscuits, cake, chocolate and chocolate sauce, etc. Some toxic compounds like hydroxymethylfurfural (HMF) are formed during production of caramel. Therefore, decrease of HMF content of the caramel is important for human health. In the present study, different dietary fibres (inulin types, wheat, apple, lemon, pea and oat fibres) were added to the caramel formulation to reduce HMF content. In addition to the HMF content of the model caramel samples, physicochemical properties such as colour, pH, brix and  $a_w$  values of the samples were determined. Both of the HMF content and physicochemical properties were significantly affected by fibre addition (*P*<0.05). High performance (HP) and high performance for high temperature process (HPX) type inulins were convenient for incorporation when considering the HMF content and colour properties of the samples. Principal component analysis was also applied to correlate variables. PC1 and PC2 explained approximately 85.58% of the variation in the data set. According to the result of this study, HP and HPX inulin samples might be added to caramel formulation or acid and sugar containing products to decrease HMF content.

Keywords: principal component analysis, sugar, acid, caramelisation, HMF

### 1. Introduction

Caramel is substantially found in the formulation of many food products. Caramel is widely used as a food additive in the food industry to improve colour and flavour of foods or beverages (Ratsimba et al., 1999) such as chocolate, cola, ice cream, biscuits, cakes, and desserts. Caramel aroma is formed through caramelisation reaction occurred by heating of sugar above 120°C in dry or in concentrated sugar solution under acidic condition (Ratsimba et al., 1999). Although this reaction is very important for the food industry, it causes formation of some undesirable compounds like hydroxymethylfurfural (HMF) formed during heat treatment applied to foods under acidic conditions as a result of dehydration of sugars (Kroh, 1994) and HMF is used as an indicator of caramelisation degree of food products (Berry and Tatum, 1965; Lee and Nagy, 1988; Pous et al., 1991). Food products including caramel in their formulations had high content of HMF. Brenna *et al.* (2009) reported that HMF content of the soft drink was found between 0.8-2 mg/l, which could be resulted from addition of caramel. In the other study, 9.5 g/kg HMF was determined in caramel products (Bachmann *et al.*, 1997). The HMF formation depends on the sugar type (Lee and Nagy, 1990), on pH (Gökmen *et al.*, 2007), and on water activity (Gökmen *et al.*, 2008). HMF is an intermediate product of caramelisation reaction and it is important for aromatic and antioxidative properties of the foods (Pous *et al.*, 1991; Shaw *et al.*, 1967; Tatum *et al.*, 1967). However in literature, there are controversial results about the toxicological relevance of HMF (Janzowski *et al.*, 2000; Lee *et al.*, 1995).

HMF is accepted as an undesirable compound in the worldwide since many studies have reported toxic effects of HMF. A high concentration of HMF is considered as

cytotoxic, irritating to eyes, upper respiratory tract, skin and mucous membranes (Capuano and Fogliano, 2011). It has been investigated that HMF induces and promotes aberrant crypt foci (preneoplastic lesions) in rat colon (Archer et al., 1992; Bruce et al., 1993). It has been also reported that HMF is a weak carcinogen in multiple intestinal neoplasia mice and it causes to increase remarkably the number of small intestine adenomas (Svendsen et al., 2009). Moreover, HMF damages to enzyme activation of cells and to functions of the liver (Archer et al., 1992; Glatt et al., 2005; Janzowski et al., 2000). HMF is converted to sulfoxymethylfurfural (SMF) by sulfotransferases in vitro and in vivo and this compound initiated tumour growth in mice skin (Surh and Tannenbaum, 1994) and it causes papilloma's formation with higher activity than HMF (Surh et al., 1994). In addition SMF was considered as mutagenic for bacterial and mammalian cells (Glatt and Sommer, 2006). Due to these reasons, HMF is accepted as a toxic compound and therefore, HMF content of many different food products has been determined by several researchers (Ameur et al., 2007; Dogan et al., 2005; Goksel et al., 2012; Murkovic and Pichler, 2006; Rada-Mendoza et al., 2004; Oral et al., 2012; Zappalá et al., 2005) and many studies about lowering HMF content of the foods or model foods have been carried out. The researchers suggested that fibre content might affect HMF uptake (Capuano and Fogliano, 2011). Therefore, addition of dietary fibre to caramel formulation might be important for both the functionality and HMF content of the caramel and products including caramel in their formulations. In addition, observation of the effect of dietary fibre is also important for products such as fruit gels and jams, including both of the sugar and acid in their formula, since there is an increasing trend in the food industry to increase functionality of the product by incorporation of the fibres.

As known, dietary fibres are widely used in food formulations since they have health-promoting effects (Gurmeric *et al.*, 2012). In addition to promote healthy eating habits in young children, dietary fibres are important for bacterial colonisation of the gastrointestinal zone (Edwards and Parrett, 2003). Dietary fibres inhibit obesity since they promote a feeling of fullness (Hanley *et al.*, 2000) and protect against several diseases like cardiovascular disease, diabetes and colon cancer (Lee *et al.*, 1992). Due to these beneficial effects, there have been many studies about the enrichment of some foods or model systems with dietary fibres (Brennan and Tudorica, 2007; Cardarelli *et al.*, 2008; Dello Staffolo *et al.*, 2004; Dogan *et al.*, 2012; Gurmeric *et al.*, 2012; Henelly *et al.*, 2006; Koca and Metin, 2004; Toker *et al.*, 2013; Yildiz *et al.*, 2013). We have not encountered any study about the effect of the dietary fibres on the HMF content and physicochemical properties of the caramel sample or similar products.

The present study aimed at determination of the physicochemical characteristics and HMF content of the caramel samples enriched with different dietary fibres. The results of the study might also provide beneficial information about the effects of the dietary fibre addition on the HMF content of the products including sugar and acid.

### 2. Materials and methods

### Materials

Inulin fibres (standard (ST) inulin, granulated (GR) inulin, high performance (HP) inulin, high performance (HPX) inulin for high temperature process, high soluble (HSI-t) inulin) were obtained from Orafti (Wijgmaal, Belgium). The other fibres (apple, oat, pea and lemon) were procured from Herbafood (Werder, Germany). Table 1 presents information, obtained from the producing company, about the composition of the inulin fibres. Moreover, Figure 1 shows the composition of the soluble and insoluble parts of the apple, oat, pea and lemon fibres. HPLC grade acetonitrile, citric acid and acetic acid were procured from Sigma (München, Germany).

### Preparation of model caramel samples

Five gram of sucrose was mixed with 0.1 g of citric acid and 0.5 g fibre. 5 ml of water was added to this mixture which was heated by drying oven (Memmert, Germany)

#### Table 1. Properties of the inulin fibres.

Inulin type	Inulin content (%)	Sugar content (%)	Mean degree of polymerisation	Sweet (%)
Standard	92	8	≥10	10
Granulated	92	8	≥10	10
High performance	100	0	>23	0
High performance for high temperature process	100	0	≥23	0
High soluble	86	14	<10	20



Figure 1. Soluble and insoluble contents of the dietary fibres (1 = apple fibre, 2 = oat fibre, 3 = pea fibre, 4 = lemon fibre).

at 130  $^\circ\!\mathrm{C}$  for 1 hour. The prepared model caramel samples were cooled to room temperature prior to the analysis.

# Determination of physicochemical properties of the samples

The soluble solid content (Brix) of the samples was determined using an automatic refractometer (Reichert AR700 Automatic Digital Refractometer; Reichert Technologies, New York, NY, USA) at room temperature and the results were expressed as °Brix at 25 °C. The pH values of the samples were determined at 25 °C by a pH meter (WTW-Inolab Level 3 Terminal; WTW-Inolab, Weilheim, Germany). The water activity ( $a_w$ ) values of the caramel samples at 25 °C were determined using an Aqualab  $a_w$  meter (Decagon, Pullman, WA, USA). The colour parameters of the samples (L, a and b values) were measured using a colorimeter (Lovibond RT Series Reflectance Tintometer; Lovibond, Amesbury, UK). Browning index values (BI) of the model caramel samples were calculated using the following equations (Maskan, 2001):

$$BI = \frac{[100(x - 0.31)]}{0.17}$$
(1)

where

$$x = \frac{(a + 1.75L)}{(5.645L + a - 3.012b)}$$
(2)

All the measurements were made in triplicate.

# Determination of hydroxymethylfurfural contents of caramel samples

The HMF compound was extracted from the samples according to the method of Fallico *et al.* (2004). A 0.25% (w/v) caramel solution was prepared (0.25 g caramel sample was weighted and it was fulfilled to 100 ml with distilled water). The solution was centrifuged (Hettich Universal 320R; DJB Labcare Ltd., Newport Pagnell, UK) at 9,000 rpm for 10 minutes and then supernatant was filtered on

0.45  $\mu$ m filter and injected into an HPLC (Agilent 1100 Series HPLC Value System; Agilent, Santa Clara, CA, USA) equipped with a diode array and multiple wavelength detector. A C18 column (ACE-15×4.6 mm, 5  $\mu$ m) was used for determination of the HMF content of the model caramel samples. The modified HPLC conditions reported by Ameur *et al.* (2006) were the following: mobile phase, 95% acetic acid solution (1% acetic acid in ultra-pure water) and 5% acetonitrile; flow rate, 1 ml/min; injection volume, 20  $\mu$ l. All the solvents were of HPLC grade (Merck, Milan, Italy). The wavelength was 284 nm. The amount of HMF was determined using an external calibration curve.

#### Statistical analysis

Classification of the model caramel samples based on the physicochemical properties and HMF content was performed by principal component analysis (PCA) using XLSTAT Software (XLSTAT 2008; Addinsoft, New York, NY, USA). PCA is a multivariate statistical technique that reduces the overall set of original variables into a smaller mathematical constructs known as principle components (Brito *et al.*, 2006). In addition, correlations among the physicochemical parameters were determined using Pearson correlation by performing XLSTAT Software (XLSTAT 2008). The SPSS package software 17.0 (SPSS Statistics, Armonk, NY, USA) was used to conduct an ANOVA to find out if the effect of different dietary fibre addition on the physicochemical properties and HMF content of the samples was significant.

### 3. Results and discussion

Colour properties (L, a and b values) and BI values, calculated using Equation 1 and 2, of the caramel samples are listed in Table 2. As can be seen, L, a and b values of the samples were in the range of 8.28-24.35, -0.95-6.71 and -0.85-13.33, respectively. Generally, incorporation of the dietary fibres significantly affected colour properties of the caramel samples (P < 0.05). As known non-enzymatic browning is associated with carbohydrate degradation occurred by Maillard and caramelisation reactions (BeMiller and Whistler, 1996). The caramelisation reaction is influenced from several factors like pH, impurities (salts) and sucrose concentration of the solution (Clarke et al., 1997; Eggleston and Vercellotti, 2000). In the present study, in order to determine effect of fibre type on the physicochemical and HMF content of the caramel samples, identical amounts of sucrose and acid were used for caramel production. From that reason, the variation in colour parameters was independent of sucrose and acid. Impurities of the fibres might cause colour differences among the samples. As known, the first step of caramelisation reaction is the conversion of the sucrose to glucose and fructose, which is followed by further degradation to other compounds (Clarke et al., 1997; Eggleston et al., 1996; Kroh,

Sample	L	а	b	Browning index
Control (no fibre)	8.28±0.05 <sup>h</sup>	-0.60±0.04 <sup>d</sup>	-0.31±0.02 <sup>d</sup>	-8.79
Standard type inulin	11.36±0.11 <sup>e</sup>	-0.66±0.02 <sup>de</sup>	-0.32±0.03 <sup>de</sup>	-6.88
High soluble type inulin	8.78±0.10 <sup>9</sup>	-0.44±0.01°	-0.36±0.05 <sup>de</sup>	-7.49
High performance type inulin	9.63±0.05 <sup>f</sup>	-0.70±0.01 <sup>f</sup>	-0.56±0.01 <sup>fg</sup>	-10.69
Granulated type inulin	11.18±0.12 <sup>e</sup>	-0.61±0.01 <sup>d</sup>	-0.45±0.02 <sup>ef</sup>	-7.74
High performance for high temperature	8.87±0.07 <sup>g</sup>	-0.65±0.02 <sup>de</sup>	-0.72±0.02 <sup>h</sup>	-12.77
process type inulin				
Wheat fibre	11.13±0.16 <sup>e</sup>	-0.95±0.02 <sup>g</sup>	-0.64±0.02 <sup>gh</sup>	-11.55
Apple fibre	17.00±0.30 <sup>c</sup>	6.71±0.08 <sup>a</sup>	13.33±0.10 <sup>a</sup>	160.66
Lemon fibre	24.35±0.21 <sup>a</sup>	-0.58±0.04 <sup>d</sup>	1.56±0.08 <sup>c</sup>	4.71
Pea fibre	23.11±0.11 <sup>b</sup>	-0.28±0.01 <sup>b</sup>	3.60±0.02 <sup>b</sup>	15.62
Oat fibre including caramel samples	12.00±0.09 <sup>d</sup>	-0.91±0.01 <sup>g</sup>	-0.85±0.02 <sup>j</sup>	-12.05

Table 2. Colour values of the caramel sa	mples including	different dietary fibres. <sup>1</sup>
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<sup>1</sup> Different superscript letters in the same row show significant differences (P<0.05).

1994) of which some are responsible of colour pigment formation. While the fibre was added to sucrose solution, degradation of fibre can occur by means of heat and acid, which cause the formation of different compounds and decrease the degradation of fructose and glucose. The decrease of amount of acid per unit sucrose concentration in the caramel system is due to the incorporation of the fibre. Therefore, formation of the different compounds by degradation of the fibres and different degradation levels of sucrose, glucose or fructose might be factors affecting colour properties of the caramel samples. In addition, pigment compounds of the fibres might also have influenced the magnitudes of the L, a and b parameters. Inulin and sugar content of the inulin fibres, sugar type found in the composition, degree of polymerisation of the inulin fibres (Table 1), might also be important factors affecting colour properties of the caramel samples including different inulin types in their formulation. As can be seen from Table 2, it seems that colour properties of the samples including inulin type fibres were very similar to the control sample, indicating that these fibres enriched caramel samples might be added to food products to improve colour. BI values of the caramel samples varied between -12.77 and 160.66. The possible factors influencing colour properties also affect the magnitude of the BI since it was calculated using L, a and b values (Equation 1 and 2). The development of the brown colour is attributed to the production of polymeric products, HMF and furfural which are reported as precursors of such polymers (Kroh, 1994; Quintas et al., 2007).

Table 3 shows the pH values of the caramel samples. As seen, the magnitude of the pH changed between 2.21 and 2.93. As pH values of the inulin including caramel samples were very close to that of control sample, caramel samples

containing apple, lemon and pea fibres had higher pH value than the control sample. The impurities and compositions of the fibres and buffering capacity of those compounds might affect pH value of the caramel samples. Brix values of the caramel samples ranged between 33.46 and 37.01. The fibre including samples except for lemon fibre had higher brix value than that of control sample, which was expected, since addition of fibre into the solution increased the soluble content of the solution. As can be seen, brix values of the inulin fibre including caramel samples were very close to each other. The other fibre including samples had lower brix value than that of inulin containing caramel samples, since the soluble composition of the other fibre types (Figure 1), was lower than those of the inulin fibres. Another parameter shown in Table 3 is the  $a_w$  value. The magnitudes of the a<sub>w</sub> values of the samples varied between 0.933 and 0.950.

HMF content of the caramel samples including different fibres were in the range of  $7.05-97.76 \,\mu\text{g/kg}$  (Table 3). Table 3 shows that addition of fibre significantly affected the HMF formation during production of caramel (P<0.05). HMF content of the wheat, apple, lemon, pea and oat containing samples had lower HMF content than that of the control sample, which is mainly associated with the insoluble part of the those fibres. The insoluble part of the fibre decreased HMF concentration, since acid cannot affect degradation of that part. The ST type inulin including caramel sample had the highest HMF content, which was followed by HSI-t and GR type inulin caramel samples. ST, HSI-t and GR type inulin caramel samples had higher HMF content than control sample, which might be resulted from the sugar content of the ST, GR and HSI-t type of the inulin. As can be seen from Table 1, ST, GR and HSI-t inulin samples included sugar in concentration of 8, 8 and 14%,

HMF (µg/kg)	) pH Soluble solid content (°Brix)		Water activity
77.61±1.51 <sup>c</sup>	2.21±0.00 <sup>j</sup>	33.54±0.02 <sup>h</sup>	0.933±0.002 <sup>e</sup>
97.76±1.59 <sup>a</sup>	2.24±0.00 <sup>ij</sup>	36.62±0.01 <sup>cd</sup>	0.934±0.001 <sup>de</sup>
85.46±1.02 <sup>b</sup>	2.26±0.01 <sup>h</sup>	36.90±0.02 <sup>ab</sup>	0.936±0.001 <sup>de</sup>
60.83±1.27 <sup>d</sup>	2.28±0.01 <sup>gh</sup>	36.42±0.01 <sup>d</sup>	0.939±0.000 <sup>cde</sup>
88.02±0.81 <sup>b</sup>	2.28±0.01 <sup>gh</sup>	37.01±0.02 <sup>a</sup>	0.939±0.001 <sup>cde</sup>
61.44±2.33 <sup>d</sup>	2.30±0.03 <sup>fg</sup>	36.80±0.01 <sup>bc</sup>	0.940±0.000 <sup>bcd</sup>
28.48±0.82 <sup>f</sup>	2.32±0.01 <sup>e</sup>	34.28±0.06 <sup>g</sup>	0.945±0.000 <sup>ab</sup>
36.40±2.10 <sup>e</sup>	2.57±0.01°	35.29±0.01 <sup>e</sup>	0.949±0.001 <sup>a</sup>
28.35±0.95 <sup>f</sup>	2.60±0.01 <sup>b</sup>	33.46±0.02 <sup>h</sup>	0.950±0.001 <sup>a</sup>
7.05±0.98 <sup>g</sup>	2.93±0.01 <sup>a</sup>	34.39±0.219	0.945±0.001 <sup>ab</sup>
38.63±1.22 <sup>e</sup>	2.38±0.02 <sup>d</sup>	34.68±0.09 <sup>f</sup>	0.944±0.003 <sup>abc</sup>
	HMF (μg/kg) 77.61±1.51° 97.76±1.59 <sup>a</sup> 85.46±1.02 <sup>b</sup> 60.83±1.27 <sup>d</sup> 88.02±0.81 <sup>b</sup> 61.44±2.33 <sup>d</sup> 28.48±0.82 <sup>f</sup> 36.40±2.10 <sup>e</sup> 28.35±0.95 <sup>f</sup> 7.05±0.98 <sup>g</sup> 38.63±1.22 <sup>e</sup>	HMF (μg/kg)         pH           77.61±1.51°         2.21±0.00 <sup>j</sup> 97.76±1.59 <sup>a</sup> 2.24±0.00 <sup>jj</sup> 85.46±1.02 <sup>b</sup> 2.26±0.01 <sup>hi</sup> 60.83±1.27 <sup>d</sup> 2.28±0.01 <sup>gh</sup> 88.02±0.81 <sup>b</sup> 2.28±0.01 <sup>gh</sup> 61.44±2.33 <sup>d</sup> 2.30±0.03 <sup>fg</sup> 28.48±0.82 <sup>f</sup> 2.32±0.01 <sup>e</sup> 36.40±2.10 <sup>e</sup> 2.57±0.01 <sup>c</sup> 28.35±0.95 <sup>f</sup> 2.60±0.01 <sup>b</sup> 7.05±0.98 <sup>g</sup> 2.93±0.01 <sup>a</sup> 38.63±1.22 <sup>e</sup> 2.38±0.02 <sup>d</sup>	HMF ( $\mu$ g/kg)pHSoluble solid content (°Brix)77.61±1.51°2.21±0.00j33.54±0.02h97.76±1.59°2.24±0.00j36.62±0.01cd85.46±1.02b2.26±0.01hi36.90±0.02ab60.83±1.27d2.28±0.01gh36.42±0.01d88.02±0.81b2.28±0.01gh37.01±0.02°61.44±2.33d2.30±0.03fg36.80±0.01bc28.48±0.82f2.32±0.01°34.28±0.06936.40±2.10°2.57±0.01°35.29±0.01°28.35±0.95f2.60±0.01b33.46±0.02h7.05±0.98g2.93±0.01°34.39±0.21938.63±1.22°2.38±0.02d34.68±0.09f

Table 3. Hydroxymethylfurfural (H	MF) content, pH, soluble solid content and	d water activity values of the caramel samples. <sup>1</sup>
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<sup>1</sup> Different superscript letters in the same row show significant differences (P<0.05).

respectively. Therefore addition of those fibres caused to increase sugar content of the solution, which might increase HMF formation during heating process since the first step of the caramelisation reaction is sucrose hydrolysis leading to production of glucose and fructose (Clarke et al., 1997; Eggleston et al., 1996; Lowary and Richards, 1988; Richards, 1986) and further degradation of these sugars is responsible for the formation of other compounds like HMF (Antal et al., 1990; Clarke et al., 1997; Eggleston et al., 1996; Kroh, 1994; Lowary and Richards, 1988; Mauch, 1971). Therefore, the increase of sugar concentration in the solution caused to increase in HMF formation. When the sugar concentration of the solution is considered, it was expected that HSI-t type including sample had higher HMF content than that of ST and GR type inulin containing samples. However, the ST type inulin including sample had a higher HMF content than that of the HSI-t type inulin containing sample, which might be associated with the sugar type found in the fibre. Lee and Nagy (1990) reported that HMF formation is dependent on the sugar type. The degradation mechanism or the degradation product of sugars under different conditions could change (Gökmen et al., 2008), which might directly affect HMF formation. Ameur et al. (2007) investigated the effect of sugar type and cooking temperature on the HMF content of the model biscuit. They found that the biscuit sample including fructose had higher HMF content than those containing glucose and sucrose when cooked at 200 °C (Ameur et al., 2007). However while the cooking temperature is 250 and 300 °C, sucrose containing samples had a higher HMF content than samples containing fructose and glucose in their formulation (Ameur et al., 2007). As seen, the HMF formation mechanism is not stable and it depends on many factors like sugar type and temperature. Another factor affecting HMF formation might be time at which HMF is formed, since HMF is degraded to levulinic and formic acid under acidic media (Amarasekara et al., 2008), causing a decrease in HMF content of the sample. HP and HPX including caramel samples had lower HMF content than that of control sample, which might have resulted from the decrease of acid concentration per unit sucrose used for degradation of sugar as some of the acid might have been used for degradation of inulin. Regarding toxic effect of HMF, inulin (HP and HPX inulin types) including caramels might be added to food products considering health aspects. In addition, it is known that many foods including sugar and acid in their formula are present in the food industry. The heating process causes HMF formation, which might be prevented by addition of inulin. Therefore, addition of inulin to media including sugar and acid is important for health due to a decreasing HMF content as well as a prebiotic effect.

# Application of PCA analysis on physicochemical properties and HMF content of the caramel samples

The use of the fibre in the formula is possible if it does not badly affect the product quality in terms of sensory properties such as colour and aroma. In order to determine which fibre including sample has similar characteristic with control sample, PCA was applied to correlate the samples with respect to physicochemical properties and HMF content. The PCA was performed to data involving physicochemical properties and HMF content of the samples to reduce and correlate variables. According to Kaiser's rule, PC1 and PC2 are adequate for description of the variance in the data set due to their eigenvalues higher than 1 (Table 4). The eigenvalue of PC1 and PC2 were 4.781 and 2.066, respectively. PC1 and PC2 accounted

Principal component	Eigenvalue	Variance (%)	Cumulative variance
1	4.781	59.761	59.761
2	2.066	25.823	85.584
3	0.591	7.389	92.974
4	0.371	4.640	97.613
5	0.184	2.294	99.907
6	0.007	0.083	99.990
7	0.000	0.005	99.996
8	0.000	0.004	100.000

Table 4. Results of principal component analysis analysis using data of physicochemical properties of the caramel samples.

for 59.761 and 25.823% (Table 4), respectively indicating that PC1 described more variability than PC2. Both of the PC1 and PC2 explained approximately 85.58% variance in the data set, which is sufficient for qualitative purposes due to higher value than 70% (Larrigaudiere et al., 2004). The other six PCs had eigenvalue lower than 1 indicating that they cannot explain adequate variance in the data set. After determination of the PCs, determination of which parameters are responsible for explanation of the variance in the PCs is important. Loadings of the PCs is presented in Table 5. According to this table, the variation in the PC1 is explained by the HMF content, pH, L, b, a<sub>w</sub> and BI values and a value explained the variation of PC2.

The score plot of PC1 versus PC2 is presented in Figure 2 providing information about the correlation coefficient between the variables which is equal to the cosine of the angle between respective vectors of the variables on the plot (Shin et al., 2010). The correlation matrices of the variables are shown in Table 6. The angle between HMF content and L, pH, a<sub>w</sub> values were approximately equal to 180°, which indicates that the correlation coefficient between these variables was very close to -1 (cos180° =



Figure 2. Plot of the first two principal component (PC) loading vectors. BI = browning index; a, b and L = colour parameters; aw = water activity; Brix = soluble solid content (°Brix); 5-HMF = hydroxymethylfurfural.

-1). As seen from Figure 2, the angle between brix value and BI, a, b values were approximately 90°, showing that no correlations were observed between these variables (cos 90° = 0). The correlation coefficient between BI value and a, b values were approximately 1, which is shown by the angle between BI and a values or BI and b values which were close to 0° of which the cosine is equal to 1. The correlation coefficient between HMF and BI value was approximately 0, which is unexpected, since the brown colour is associated with HMF and furfural as mentioned above (Kroh, 1994). Therefore there are many factors affecting this result which were discussed in the previous section.

As can be seen in Figure 3, control, ST, HSI-t, HP, GR and HPX type inulin samples were clustered together on the upper left quadrant of the plot. The HMF content and brix values of the caramel samples resulted in this clustering.

able 5. Loadings of the significant principal components (PC) and contributions of components.								
Component	PC1	Contributions (%)	PC2	Contributions (%)	PC3	Contributions (%)		
Hydroxymethylfurfural	-0.805	13.561	0.477	11.007	0.046	0.352		
bH	0.845	14.919	-0.323	5.044	0.318	17.119		
-	0.822	14.140	-0.379	6.968	0.255	11.013		
a	0.681	9.687	0.724	25.359	-0.112	2.110		
0	0.830	14.419	0.547	14.505	-0.035	0.209		
Soluble solid content (°Brix)	-0.514	5.531	0.552	14.736	0.630	67.232		
Nater activity	0.867	15.727	-0.213	2.188	0.062	0.661		
Browning index	0.758	12.015	0.646	20.192	-0.088	1.306		

Most significant loadings are given in bold.

	HMF	рН	L	а	b	Brix	a <sub>w</sub>	BI
HMF	1							
pН	-0.807	1						
L	-0.716	0.905	1					
а	-0.212	0.304	0.252	1				
b	-0.395	0.539	0.480	0.964	1			
Brix	0.651	-0.443	-0.523	-0.019	-0.161	1		
a <sub>w</sub>	-0.852	0.702	0.755	0.434	0.558	-0.460	1	
BI	-0.301	0.408	0.362	0.993	0.988	-0.093	0.506	1

Table 6. Correlations among the parameters of the caramel samples.

HMF = hydroxymethylfurfural; a, b and L = colour parameters; Brix = soluble solid content (°Brix);  $a_w$  = water activity; BI = browning index. Significant values (except diagonal) at the significant level of alpha=0.050 (two-tailed test) are given in bold.



Figure 3. Plot of the first two principal component score vectors (S1 = control (no fibre); S2 = ST type inulin; S3 = HSI-t type inulin; S4 = HP type inulin; S5 = GR type inulin; S6 = HPX type inulin; S7 = wheat fibre; S8 = apple fibre; S9 = lemon fibre; S10 = pea fibre; S11 = oat fibre including caramel samples).

The location of the wheat, lemon, pea and oat fibre caramel samples was on the lower right quadrant of the plot due to its high a, b and BI values which were very high when compared with those of the other samples. Regarding colour properties, wheat, lemon, pea and oat fibres might not be used in the formulation of caramel sample or samples containing sugar and acid since colour of the product is important factor affecting attractiveness of the product.

## 4. Conclusions

Caramel is widely used in food industry in order to improve colour and flavour of the products like chocolate, biscuits, cakes, desserts and ice cream. However caramel includes undesirable compounds like HMF that are formed during the heating process or storage period. From that reason a decrease of HMF content is important for human health since that compound has toxic effects. In recent years, dietary fibres are added to the food product formulations to improve functional properties of the products as consumers are aware of relation between diet and health. In the present study, HMF content of the model caramel products enriched with different dietary fibres was investigated and it was observed that the addition of the some inulin types to a model caramel system decreased the HMF content of the caramel samples. The PCA was satisfactorily applied to correlate variables and classify the caramel samples based on the physicochemical properties and HMF content of the caramel samples.

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