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ORIGINAL PAPERS

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CHEMICAL COMPOSITIONS, ANTI-OXIDATIVE ACTIVITIES AND BLOOD GLUCOSE LOWERING PROPERTIES OF RAW AND FERMENTED AFRICAN STAR APPLE SEEDS (*CHRYSOPHYLLUM AFRICANUM*) FLOUR

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ABSTRACT

Plant-based foods have health benefits and minimal side effects than synthetic anti-diabetic agents. Therefore, this study evaluated nutritional and anti-hyperglycaemic efficacies of raw African star apple (RASA) and fermented African star apple seeds (FASA). The RASA and FASA seeds were processed into flour, and nutritional qualities, anti-oxidative and antidiabetic activities in Wistar rats were evaluated at different concentrations (i.e., 20, 30, 40% ASA) respectively. Crude protein (g/100g) and energy values (kcal/100g) of RASA and FASA ranged as follows: 11.8 - 23.9 and 372.1 - 417.5 respectively. The Na/K (1.11 – 1.72) and Ca/P (12.71 – 18.71) molar ratios of RASA and FASA samples were greater than recommended values of <1.0 and >1.0 respectively. Total essential amino acids of RASA and FASA were 33.14 and 48.48 g/100g of protein respectively, while predicted protein efficiency ratio, essential amino acid index and biological value were 2.25 – 3.29, 58.43 – 83.68% and 51.99 – 79.51% respectively. Tryptophan and methionine were the first and second limiting amino acids in RASA and FASA respectively. FASA had higher free radical scavenging activities in DPPH, FRAP and metal chelation inhibitory activities than in RASA. Blood glucose lowering effects of RASA and FASA in diabetic-induced rats increased as the concentration of ASA increased (i.e., 89% $RASA_{40\%}$, 78% $FASA_{40\%}$). In conclusion, the African star apple seeds could be valuable and excellent source for low-priced functional foods, and the seeds are characterized by essential nutrients, crude fiber, ability to scavenge free radicals and blood glucose lowering potentials.

KEYWORDS:
Nutritional compositions, Antioxidative activities, Anti-hyperglycaemic potentials

INTRODUCTION

Plants have been used since antiquity for food and medicinal purposes by diverse people and cultures throughout the world. The use of plants as traditional health remedies is very popular in many parts of developing countries than ever before, because they have the potential of myriad benefit to the society or indeed to the entire mankind especially in the line of nutrition, medicine and pharmacology [1]. Besides, medicinal plants have been reported to have minimal side effects [2] when compared with synthetic drugs [1]. In view of this, researches have been focused on scientific evaluation of traditional drugs of plant origin to manage diseases like diabetes in the last decade [1]. The medicinal value of plants lie in the bioactive components such as phytochemical, protein and fiber that produce definite physiological action on the human body [3].

Diabetes mellitus is a heterogeneous group of metabolic diseases that is characterised by hyperglycaemia. Persistent hyperglycemia in diabetes leads to metabolic dysfunction and manifests in form of series of complications like blindness, kidney and heart disease, stroke, loss of limbs, and reduced life expectancy if left untreated [4]. Oral hyperglycaemic agents has shortcomings such as ineffectiveness in oral administration, short shelf life and in the event of excess dosage fatal hypoglycaemia may occur which limit its usage [5]. Hence, seeking natural and non-toxic anti-diabetic agents in plants like African star apple seeds is necessary for diabetic therapy.

African star apple (*Chrysophyllum africanum*) is a wild plant and popularly called "Agbalumo", "Agwaluma" or "Udara" among the Yoruba, Hausa and Igbo tribes respectively. It belongs to the family of *Sapotaceae,* and widely grown in many parts of tropical regions of the world [6]. The fleshy pulp of African star apple fruits are usually consumed fresh as snack and also used in food industries to produce jam, jellies, and also, the seeds could be incorporated with cereals to formulate diets that are suitable for both animal and human consumption without any side effects [7].

MATERIALS AND METHODS

Sources of Food Materials and Wistar rats. Freshly harvested African apple star fruits were bought from Owena market in Ondo State. The fruits were identified and authenticated at Herbarium Unit of Department of Crop Production and Pest, Federal University of Technology, Akure, Nigeria. The Wistar albino rats were purchased from Central Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria. The study protocol was approved by the Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Akure, Nigeria (Ref. No: FUTA/2017/045).

Processing of African Star Apple Seed Flour. The seeds were removed from the fruits, sorted, manually shelled, washed with double distilled water and drained. The drained seeds were divided into two portions. One of the portions (2 kg) was blanched at 100 ºC for 45 min, drained and wrapped with plantain leaves to ferment naturally for three days using local method, while the second portion (2 kg) was treated as raw. The unfermented and fermented seeds were oven dried at 60 ºC (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain raw and fermented African star apple seed flour. The unfermented and fermented African star apple seed flour were separately packed in a plastic container sealed with an aluminium foil and stored at room temperature (~27 ºC) until required for use.

Processing of Maize flour. Raw kernels were sorted, winnowed, manually washed with double distilled water and drained. The drained popcorn kernels were blanched at 100 ºC for 40 min to enhance softening of the kernels prior to the next processing stage. The kernels were oven dried at 60 ºC (Plus11 Sanyo Gallenkamp PLC, UK) for 20 h, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain maize flour. The flour was packed in a plastic container seal with an aluminum foil and stored at room temperature (~27 ºC) until required for use.

Application of African star apple seed flour in Food formulations and its Anti-diabetes potentials. Food Formulations. Unfermented and fermented African star apple seed flours were incorporated into maize-based flour at different concentrations (20%, 30% & 40%) to obtain the following experimental food samples, that is, Raw African star apple seeds flour ($RASA_{20,}$ $RASA_{30,}$ & $RASA_{40}$) and Fermented African star apple seeds flour ($FASA_{20,}$ $FASA_{30}$ & $FASA_{40}$).

Nutritional and phytochemicals compositions of raw and fermented African seeds flours. Proximate compositions. Proximate compositions of raw and fermented African seeds flours were determined using the standard procedures of Association of Official Analytical Chemists (AOAC) [8]. Moisture content was determined in a hot-air circulating oven (Galenkamp). Ash was determined by incineration (550 °C) of known weights of the samples in a muffle furnace (Hotbox oven, Gallencamp, UK, size 3) [8]. Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60 °C) using Tecator Soxtec (Model 2043(20430001), 69, Slandegarupgade, DK-3400, Hilleroed, Denmark) [8]. Protein content (N × 6.25) was determined by the micro-Kjeldahl method (Method No 978.04) [8]. Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide [8]. Carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash and crude fibre and subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate.

% carbohydrate = 100-(% Moisture + % Fat + % Ash + % Crude fibre + % Crude protein)

The energy value of the samples was estimated [in kcal/g] by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors 4.0, 9.0 and 4.0 respectively.

Mineral Compositions. Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). Sodium (Na) and potassium (K) in the food samples were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as the standards [8]. Phosphorus was determined using Vanodo-molybdate method.

Amino Acid Compositions. The amino acid profiles of the experimental samples were determined according to the method described by AOAC [8]. The experimental samples were digested using 6N HCl for 24 h. Amino acids were determined using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method. The data were calculated as grams of amino acid per 100 g crude protein of flour sample.

Calculated Nutritional Quality. Protein efficiency ratio (PER). The protein efficiency ratio of the functional complementary foods was calculated as reported by Ijarotimi et al. [9].

P-PER = -0.468 + 0.454(Leu) - 0.105(Tyr)

Essential Amino Acid Index (EAAI). Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated as reported by Ijarotimi et al. [9].

$$EAAI\ (\%) = \mathrm{n}\sqrt{\frac{100a\ x\ 100b\ldots.100j}{av\ x\ bv\ \ldots\ldots jv}}$$

Where:

n = number of essential amino acids, a, b …..j = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine) in test sample and av, bv ….. jv = content of the same amino acids in standard protein (%) (egg or casein) respectively.

Predicted Biological Value (PBV). Biological Values were computed as reported by Ijarotimi et al. [9]. The following equation was used for BV determination.

PBV = 1.09 (EAA Index) – 11.7.

Nutritional Index (NI). The nutritional index of the food samples was calculated as reported by Ijarotimi et al. (2013) [9].

$$Nutritional\ index\ (\%) = \frac{EAAIx\ \%protein\ in\ sample}{100}$$

Phytochemicals. Determination of flavonoids was carried out by the gravimetric procedure of Harborne [10]. The quantitative determination of alkaloids was carried out by the alkaline precipitation using the gravimetric method described by Harborne [10]. Tannin concentration in the samples were determined as described by AOAC [8]. Phenols concentration was determined as described by Singleton et al. [11]. Determination of saponins content of the sample was determined by double solvent extraction gravimetric method [10]. Phytate was determined using spectrophotometric method of Lucas and Markaka [12].

Determination of Antioxidant Activities of African Star Apple Seeds Samples. DPPH radical scavenging assay. The scavenging effect of African star apple seeds flour samples on 2, 2- Diphenyl-1-picryhydrazyl (DPPH) free radical was measured according to the method of Hwang *et al*. [13]. African star apple seed flour extract (10 mg) were dissolved in 1 ml of buffer (0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100). DPPH was dissolved in methanol to a final concentration of 100 µM. Sample extracts (100 µl) were mixed with 100 µl of the DPPH solution in the 96-well plate to a final assay concentration of 1 mg/ml and incubated at room temperature in the dark for 30 min. The absorbance values of the blank, Glutathione (GSH) (control) and samples were measured at 517 nm. The control consisted of sodium phosphate buffer in place of the protein fractions sample while Glutathione (GSH) was used as the positive control. The percent DPPH radical scavenging activity of the samples were determined using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\mathrm{A}_{517}\ of\ sample}{\mathrm{A}_{517}\ of\ blanc}\right) \mathrm{X}\ 100$$

Ferrous metal ions chelating effect. The metal chelating activity of the African star apple seeds flour samples were determined using a modified method of Decker and Welch [14]. Experimental samples and Glutathione (GSH) solution (final assay concentration of 1 mg/dL) was combined with 0.05 mL of 2 mM $FeCl_2$ and 1.85 mL double distilled water in a reaction tube. Ferrozine solution (0.1 mL of 5 mM) was added and mixed thoroughly. The mixture was then allowed to stand at room temperature for 10 min from which an aliquot of 200 µL was removed and added to a clear bottom 96-well plate. A blank experiment was also conducted by replacing the sample with 1 ml of double distilled water. The absorbance of blank (Ab) and sample (As) at 562 nm were measured using a spectrophotometer and the metal chelating activity of the sample was compared to that of GSH. The percentage chelating effect (%) was calculated using the following equation:

$$\text{Metal chelating activity (\%)} = \left(1 - \frac{\mathrm{A}_{517}\ of\ sample}{\mathrm{A}_{517}\ of\ blanck}\right) \mathrm{X}\ 100$$

Nitric oxide Radical (NO) Scavenging Assay. The nitric assay was carried out as described by scientific methods [15]. The nitric oxide was generated from sodium nitroprusside (SNP). The reaction mixture (5.0 mL) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the experimental flour sample extracts at different concentrations, was incubated at 25 ˚C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The nitric oxide (NO) radical generated interacted with oxygen to produce the nitrite ion (NO) which was assayed at 30 min intervals by mixing 1.0 mL of incubation mixture with an equal amount of Griess reagent (1 % sulfanilamide in 5 % phosphoric acid and 0.1 N-(1-Naphthyl) ethylenediamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with N-(1-Naphthyl) ethylenediamine dihydrochloride) was measured at 550 nm on spectrophotometer. The nitrite generated in the presence or absence of the flour extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Calculated the % inhibition by formula and plot graph in compared to standard.

$$\text{NO inhibitory activity (\%)} = \left(1 - \frac{A_{517}\ of\ sample}{A_{517}\ of\ blanck}\right) X\ 100$$

Ferric-reducing antioxidant property (FRAP). The ferric reducing power of the protein fraction samples was determined according to the method of Mau *et al*. [16]. Experimental sample or Glutathione (GSH) was dissolved in 0.2 M phosphate buffer, pH 6.6; an aliquot (250 µL) was mixed with 250 µL of the buffer and 250 µL of 1% potassium ferricyanide solution. The mixture was thoroughly mixed using a vortex machine and heated at 50 °C for 20 min. After incubation, 250 µL of 10% trichloroacetic acid (TCA) was added followed by 50 µL of 0.1% ferric chloride dissolved in double distilled water and then 200 µL of double distilled water was added. The solution was allowed to stand for 10 min at room temperature, after which it was centrifuged at 1000 ×g for 10 min. An aliquot (200 µL) of the supernatant was transferred to a clear bottom 96-well plate and the absorbance was measured at 700 nm.

Anti-diabetes potential of fermented and unfermented African star apple seeds flour. Induction of Diabetes mellitus in rats. The baseline blood glucose levels of the animals were measured before induced with Aloxan drug. Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared solution of Aloxan monohydrate (150 mg/kg. body weight) dissolved in physiological saline in overnight fasted Wistar Albino rats [17]. The rats were allowed to drink 5% glucose solution to avoid hypoglycaemic effects of the drug. The blood glucose levels in the animals were measured 72 h after the drug administration (Aloxan treatment) through tail tipping using glucometer (Accu-Chek, Active, Roche Diagnostic's 9115 Hague road, Indianapolis, 46256 Lot No 115764) and those found to be diabetic (serum glucose ≥ 250 mg/dL) were selected for the study [18].

Anti-diabetic potentials of raw and fermented African star apple seeds flours. The diabetic induced rats were divided into seven groups containing 5 rats each, and six of the groups were fed on experimental food samples, and water *ad libitum,* while animals in the control group were fed on commercial feeds and treated with metformin hydrochloride (an antidiabetic drug), which was dissolved in their drinking water for fourteen days. The blood glucose levels of the rats were measured in the morning by drawing blood from each rat through tail tipping using Accu check® glucometer kit [19].

Statistical Analysis. The results obtained from the various analyses in triplicates were subjected to Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS) IBM Version 20.0. Coefficient of variation (CV %) was calculated to show level of significant difference.

RESULTS AND DISCUSSION

Nutritional compositions of raw and fermented African star apple seeds flour (ASA). The nutrient compositions of raw and fermented African star apple seeds flour are presented in Table 1. The moisture content of raw and fermented African star apple seeds (ASA) flour were 8.8 and 9.6 g/100g respectively, while crude fiber content showed that raw ASA (1.7 g/100g) had higher value when compared to fermented ASA flour samples (7.4 g/100g). The crude protein and energy values of the flour samples ranged as follows: 11.8 - 23.9 g/100g and 372.1 - 417.5 kcal/100g respectively. Nutritionally, it was observed in this present study that the crude protein content of fermented ASA flour sample was significantly higher, while energy value was insignificantly lower when compared to the raw ASA flour sample at coefficient of variations of 33.9% and 5.7% respectively. These findings could be attributed to the activities of microorganisms that utilised other components of the seed flour, such as carbohydrate, fat, etc. to synthesised protein or as source of energy for their biochemical activities. This finding agreed with other scientific studies who reported that fermentation have multiple effects on the nutritional value of food products like decreasing the level of carbohydrates, antinutrients [20], and also, by increasing the content of essential amino acids (lysine, methionine and tryptophan) [21]. The protein and energy values of ASA seeds flour samples in this present study were comparatively higher than the values reported by Bello and Henry [22].

TABLE 1
Formulated food samples from maize flour supplemented with African star apple (ASA)

Food Samples	Maize flour (%)	Raw ASA (%)	Fermented ASA (%)
$RASA_{20}$	80	20	-
$RASA_{30}$	70	30	-
$RASA_{40}$	60	40	-
$FASA_{20}$	80	-	20
$FASA_{30}$	70	-	30
$FASA_{40}$	60	-	40

RASA = Raw African star apple seeds flour, FASA = Fermented African star apple seeds flour

TABLE 2
Proximate compositions (g/100 g), minerals (mg/100 g) and energy values (kcal/100g) of raw and fermented African star apple seeds

Parameters	RASA	FASA	MEAN	SD	CV (%)
Moisture	8.8	9.6	9.2	0.4	4.3
Fat	9.6	7.4	8.5	1.1	12.9
Fiber	1.7	1.4	1.55	0.15	9.68
Ash	1.4	8.9	5.15	3.75	72.8
Protein	11.8	23.9	17.85	6.05	33.9
CHO	67	48.8	57.9	9.1	15.7
Energy	417.5	372.1	394.8	22.7	5.7
Mineral compositions					
Ca	210.0	212.0	211.0	1.0	0.5
Mg	141.0	149.0	145.0	4.0	2.8
K	234.0	214.0	224.0	10.0	4.5
Zn	18.1	18.9	18.5	0.4	2.2
Fe	63.7	86.0	74.85	11.15	14.9
P	16.6	11.4	14	2.6	18.6
Na	402.0	236.0	319.0	83	26.0
Pb	23.8	9.9	16.85	6.95	41.2
Cu	58.2	32.0	45.1	13.1	29.0
Mn	50.4	13.5	31.95	18.45	57.7
Na/K	1.72	1.11	1.415	0.305	21.6
Ca/P	12.71	18.71	15.71	3.0	19.1

RASA = Raw African star apple seeds flour, FASA = Fermented African star apple seeds flour

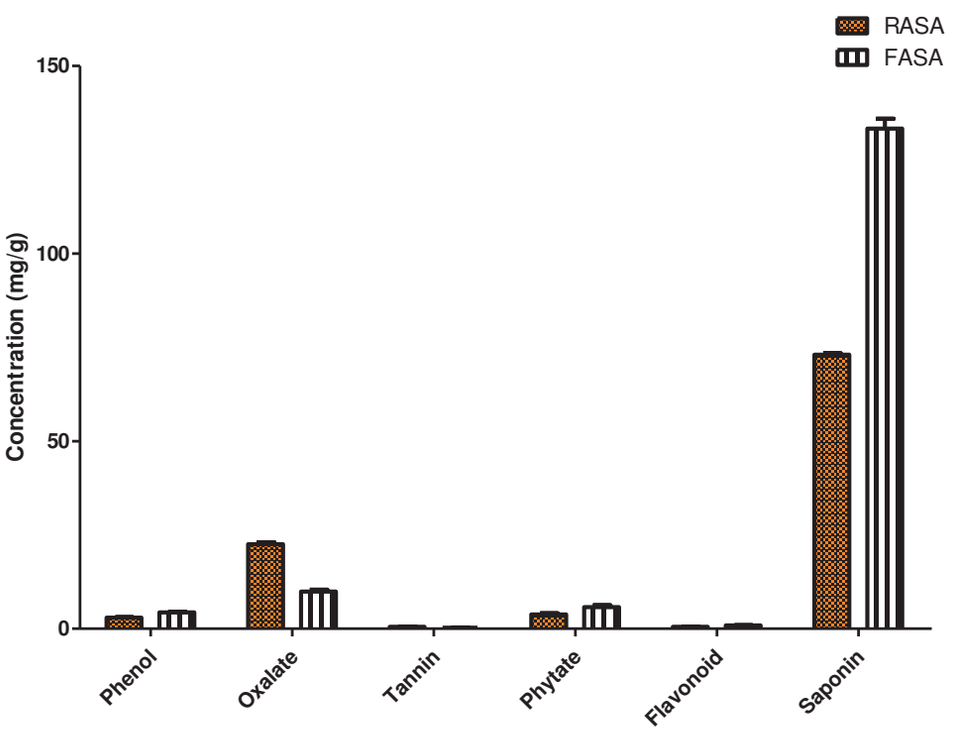

FIGURE 1
Anti-nutrient compositions of raw and fermented African star apple seeds
Key: RASA (raw African star apple flour); FASA (fermented African star apple flour)

The mineral compositions of the ASA seed flour samples were presented in Table 1. Sodium had the highest concentration (236.0 – 402.0 mg/100g), while phosphorous had the lowest concentration (11.4 - 16.6 mg/100g). The mineral compositions, i.e., Ca, Mg, K, Zn, Fe, P, Na, Pb and Cu, in raw

ASA sample were comparatively higher than that of fermented ASA sample. The high sodium content observed in this present study was agreed with the report of Bello and Henry [22]. The Na/K (1.11 – 1.72) and Ca/P (12.71 – 18.71) molar ratios of ASA samples were greater than WHO [23] recommended values of <1.0 and >1.0 respectively. This implies that ASA seeds are higher in sodium than potassium element; hence, consumption of ASA seeds may not be suitable for a patient with cardiovascular diseases. However, the high values of Ca/P molar ratios obtained in this study showed that intakes of ASA seeds might facilitate bone and teeth formations, and thereby prevent rickets in children and osteoporosis in adults. Scientific studies have reported that consumption of foods with low Na/K and high Ca/P molar ratios have some health benefits such as preventing heart diseases [24], and promoting bone and teeth formation in children [25].

The amino acid compositions of raw and fermented ASA seeds flour samples are presented in Table 2. The total essential amino acids and non-essential amino acids of raw and fermented ASA ranged as 33.14 – 48.48 and 47.68 – 50.57 g/100g of protein respectively. Glutamic acid had the concentration (14.68 – 14.99 g/100g of protein), whereas, tryptophan was observed to be in lowest concentration (0.95 – 1.18 g/100g of protein). This finding agreed with the report that glutamic acid is the most abundant amino acid in plant-based food materials [26]. The first and second limiting essential amino acid scores of ASA samples were tryptophan and methionine respectively. The predicted nutritional qualities of ASA samples showed that protein efficiency ratio (PER), essential amino acid index (EAAI), biological values (BV) and nutritional index (NI) of the experimental samples ranged as follows: 2.25 – 3.29, 58.43 – 83.68%, 51.99 – 79.51% and 6.9 – 20% respectively. Statistically, the PER, EAAI, BV and NI of fermented ASA samples were significantly ($p<0.05$) higher than raw ASA sample. This observation could be due to variation in the processing methods of ASA samples. It is well established that fermentation usually improved the nutritional qualities of fermented food products through the activities of the microorganisms [21]. Nutritionally, the total essential amino acid composition of ASA samples may be suitable to provide the essential amino acid requirements of both children and adults.

Anti-nutrient compositions of raw and fermented African star apple seeds flour (ASA). The anti-nutrient compositions of ASA samples are shown in Figure 1. The values of the anti-nutrients varied from 9.9±0.0 to 22.5±0.01 mg/g for oxalate, 0.3±0.0 to 0.6±0.0 mg/g for tannin, and 3.8±0.01 to 5.8±0.03 mg/g for phytate; while flavonoid and saponin ranged from 0.5±0.0 – 0.9±0.0 mg/g and 72.7±2.4 - 133.3±5.2 mg/g respectively. The present study showed that ASA samples were low in phytate, oxalate and tannin, but high in saponin. Scientific studies have reported that consumptions of tannin, oxalate or phytate at high dosage chelate with other vital nutrients like divalent/trivalent mineral ions (Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} & Cu^{2+}) [27] and protein [28]. In recent time, it is evident that prolong intakes of antinutrients at low concentration have some health benefits like free radical scavenging activities, lowering the risk of heart disease [29], and diabetes by reducing the rate of starch digestion and slowing gastric emptying [30].

Functional Properties of raw and fermented African star apple seeds flour (ASA). The Functional Properties of raw and fermented African star apple seeds flour (ASA) is shown in Table 2. Bulk density (BD) of the samples ranged from 0.75±0.01 mg/mL in raw ASA to 0.8±0.00 mg/mL in fermented ASA, and that these values were relatively low. It is evident that the lower the BD value, the higher the amount of flour particles that can stay together and thus increasing the energy content that could be derivable from such foods [31]. The water absorption capacity (WAC) (4.1±0.01 mL/g) of raw ASA was lower than fermented ASA sample (6.1±0.3 mL/g). This observation agreed with the report of Appiah et al. [32], who reported that the bulk density of fermented *Artocarpus altilis* seeds flour (0.57 gcm^{-3}) was significantly higher ($p<0.01$) than that of unfermented flour (0.46 gcm^{-3}). The differences in WAC between fermented ASA and raw ASA could be attributed to the denatured proteins which could bind more water through exposure of hydrophilic groups [33]. In contrary, the oil absorption capacity (OAC) of raw ASA sample (2.4±0.2 mL/g) was higher than that of fermented sample (2.1±0.0 mL/g). The foaming capacity (FC) of raw ASA sample (27.0%) was higher than fermented ASA sample (11.1%), this observation agreed with the findings of Igbabul *et al.* [34], who reported that fermentation reduced foaming capacity when compared with unfermented legumes or cereals. For the least gelation, fermented ASA sample (1.2±0.0%) was higher when compared to that of raw ASA sample (0.5±0.0%). The high gelation in fermented ASA could be attributed to the fact that fermentation mighty have denatured the ASA proteins and, thus, caused more aggregation than in the unfermented ASA flour. This observation agreed with the report of Igbabul *et al.* [34], who similarly observed that fermentation increased the least gelation concentration of coconut flour. The ability of protein to form gels and provide structural matrix for holding water, flavours, sugars and food ingredients is useful in food application and product development.

TABLE 3
Amino Acid profiles (g/100 g of protein) of raw and fermented African star apple seeds

	RASA	FASA	MEAN	SD	CV (%)	REF.
Essential amino acids						
Valine	4.23	5.42	4.83	0.60	12.33	3.5
Threonine	3.74	6.26	5.00	1.26	25.20	3.4
Isoleucine	4.47	5.09	4.78	0.31	6.49	2.8
Leucine	6.01	9.51	7.76	1.75	22.55	6.6
Lysine	6.39	9.67	8.03	1.64	20.42	5.8
Methionine	1.81	2.77	2.29	0.48	20.96	2.2
Phenylalanine	3.15	5.41	4.28	1.13	26.40	2.8
Histidine	2.39	3.17	2.78	0.39	14.03	1.9
Tryptophan	0.95	1.18	1.07	0.12	10.80	1.1
ΣEAA	33.14	48.48	40.81	7.67	18.79	30.1
Non-essential amino acids						
Arginine	4.89	6.02	5.46	0.56	10.36	2.0
Alanine	5.75	5.36	5.56	0.20	3.51	-
Serine	3.46	3.02	3.24	0.22	6.79	-
Proline	3.13	3.06	3.10	0.03	1.13	-
Glutamate	14.99	14.68	14.84	0.16	1.04	-
Glycine	3.44	3.84	3.64	0.20	5.49	-
Tyrosine	4.15	4.38	4.27	0.12	2.70	-
Aspartate	6.82	7.91	7.37	0.55	7.40	-
Cystine	1.05	2.3	1.68	0.63	37.31	-
ΣNEAA	47.68	50.57	49.125	1.445	2.94	-
Predicted Nutritional Qualities						
PER	2.25	3.29	2.77	0.52	18.77	2.5
EAAI (%)	58.43	83.68	71.06	12.63	17.77	70
Predicted BV (%)	51.99	79.51	65.75	13.76	20.93	70
Nutritional index (%)	6.9	20.0	13.45	6.55	48.70	
Essential Amino Acid Scores						
1st LAA	Tryptophan	Tryptophan	-	-	-	-
2nd LAA	Methionine	Methionine	-	-	-	-

*FAO/ WHO (1991). 1st and 2nd Limiting

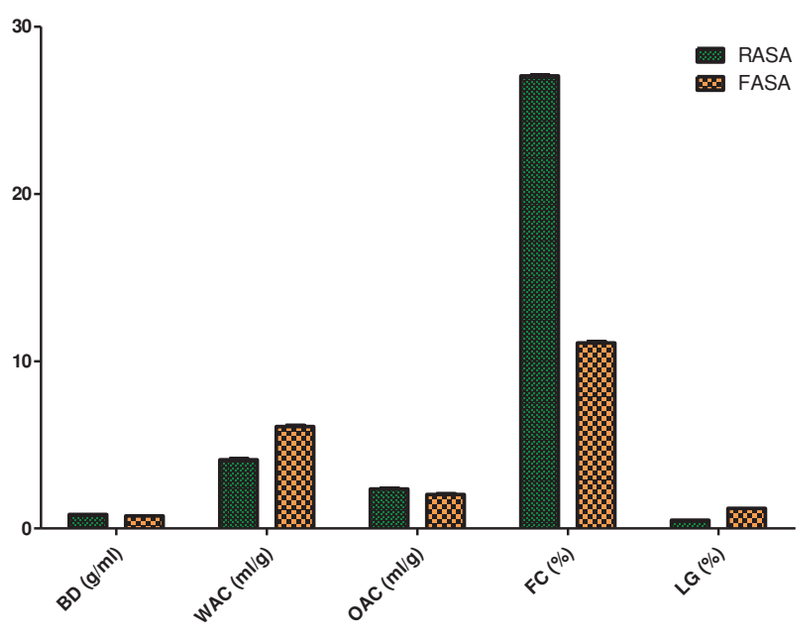

FIGURE 2
Functional properties of raw and fermented African star apple seeds
Key: RASA (raw African star apple flour); FASA (fermented African star apple flour)

Antioxidant Activities of raw and fermented African star apple seeds flour (ASA). The free radical sacvenging activities of raw and fermented African star apple seeds flour were presentd in Fig. 3. The free radical scavenging activities of raw and fermented African star apple seeds flour in 1, 1-diphenyl-2-picrylhydrazine (DPPH) showed that fermented ASA sample had the highest percentage of free radical scavenging activity (61.4±2.1%) than that of raw ASA sample (4.6±0.0%). The ferric reducing activity power (FRAP) of the samples ranged from 21.9±1.1% in raw ASA to 22.5±0.3% in fermented ASA sample, metal chelating activities varied from 45.6±1.7% in raw ASA to 57.6±3.01% in fermented ASA sample, and nitric oxide inhibitory activity ranged between 45.1±0.4% in raw and 45.3±1.3% in fermented ASA sample. The present study established that free radical scavenging activities of fermented ASA sample in DPPH, FRAP and metal chelation inhibitory activities were higher than in raw ASA seeds flour sample. This observation could be attributed to the effect of fermentation, which might have increased the antioxidative and free radical scavenging activities of fermented ASA compared to that of raw samples. This finding agreed with the report of other researcher, who reported on the effects of fermentation on antioxidative activities of plant-based foods [35]. Scientific studies have shown that fermentation improves antioxidative activities by increasing the release of bioactive compound like phytochemicals or bioactive proteins from plant-based foods, and thereby increasing the antioxidants activities [35].

Blood Glucose lowering potential of raw and fermented African star apple seeds flour. The blood glucose concentrations (mg/dL) of diabetic induced rats fed on raw and fermented African star apple seeds flour at different concentrations compared with rats administered with metformin (control) are presented in Fig. 4. The percentage reduction of blood glucose concentration of diabetic-induced rats fed on raw ASA seeds flour ranged from 45% in $RASA_{20\%}$ to 89% in $RASA_{40\%}$, while that of fermented ASA flour samples ranged from 13% in $FASA_{20\%}$ to 78% in $FASA_{40\%}$. The finding also showed that the blood glucose reducing potential of ASA increases as the concentration of the flour increased. The blood glucose reducing potentials of raw and fermented ASA flour samples in diabetic-induced rats were comparatively higher than in rats administered with metformin (a synthetic anti-diabetic drug) (41%), except in $FASA_{20\%}$. This observation showed that ASA seeds flour has the potential to reduce blood glucose concentration, hence, the seeds may be suitable as anti-diabetic agent. Comparatively, the present finding agreed with the report of other scientific study, which reported on the anti-hyperglycaemic potentials of seeds [36].

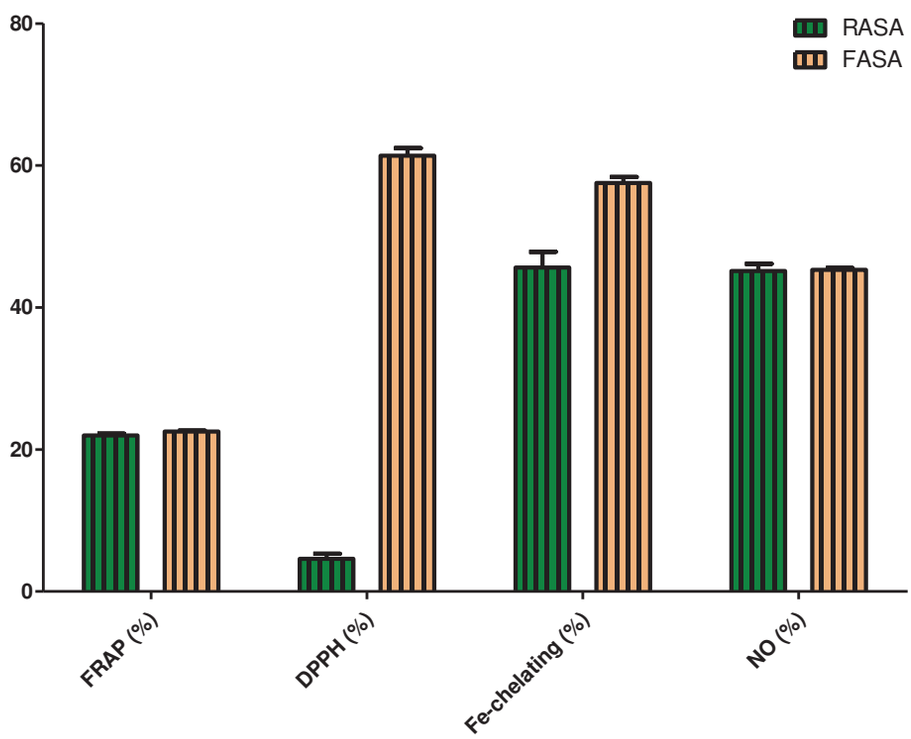

FIGURE 3
Antioxidant properties of raw and fermented African star apple seeds
Key: RASA (raw African star apple flour); FASA (fermented African star apple flour)

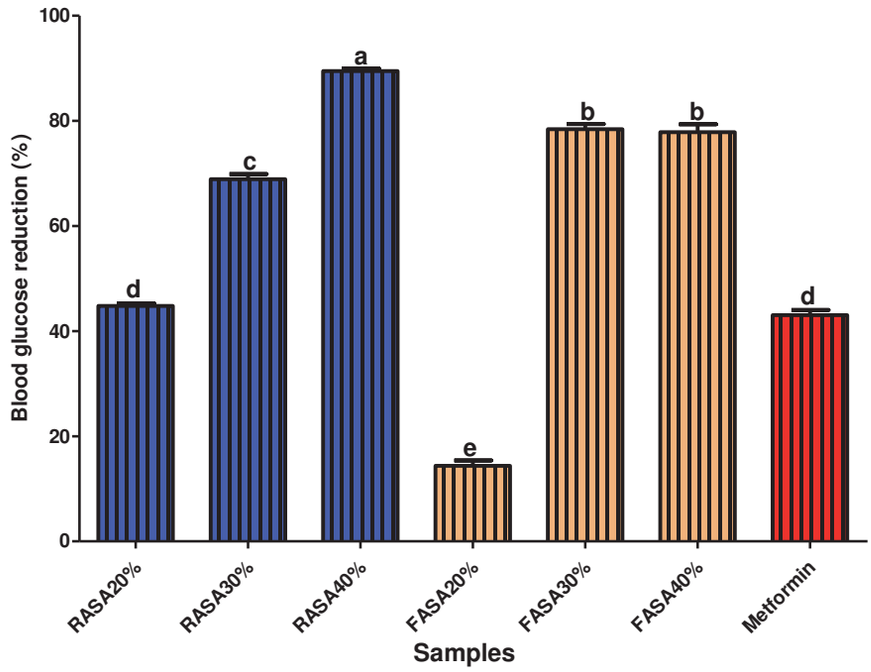

FIGURE 4
Blood Glucose reducing potential of raw and fermented African star apple seeds in rats
Key: RASA (20, 30 & 40% of raw African star apple flour); FASA (20, 30 & 40% of Fermented African star apple flour)

CONCLUSION

The present study established that African star apple seeds contain appreciable amount of essential amino acids, low antinutrient factors, antioxidant activities and anti-hyperglycaemia potentials in Wistar rats. Therefore, prolong intake of these seeds as functional food components may be suitable to manage diabetes mellitus.

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EFFECT OF INULIN AND CARRAGEENAN AS A FAT REPLACER ON PHYSICOCHEMICAL, COLOR AND SENSORY PROPERTIES OF HEATED-SAUSAGE

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ABSTRACT

The present investigation was done to study the effect of inulin and carrageenan as fat replacer on physicochemical and sensory properties of sausage. Studied treatments were including treatments with 2.92% inulin, 5.84% inulin, 2.92% carrageenan, 5.84% carrageenan, 1.46% inulin and carrageenan and 2.92% inulin and carrageenan. After preparation of samples, physicochemical, color and sensory tests were done. Removing fat from the formulation caused to increase in the moisture and reduce the amount of protein, ash, and fat. Increasing in the levels of inulin and carrageenan caused to increase in the amount of moisture. The lowest amount of fat was related to the treatment of 5.84% inulin (6.21%). Replacement caused increase in the levels of proteins. Replacement was also caused to incensement of the levels of ash. Removal of fat caused decrease in the L factor, incensement of a and decrease of b. Treatment contain 2.92% inulin and 2.92% carrageenan had the highest sensory scales but it had no significant difference with other treatments for the appearance. Its hope that measures be taken to see production of sausage samples without harmful fat using inulin and carrageenan replacements in meat products industry of country.

KEYWORDS:
Sausage, Inulin, Carrageenan, Fat replacement, Physicochemical properties, Color properties, Sensory evaluation.

INTRODUCTION

The demand for meat products with lower fat contents has increased in recent years due to new guidelines recommending reduced saturated fat intake and consumers' desire to lose weight [1, 2]. Several alternative strategies have been developed for production of low fat meat products of which substitution of saturated fat with several types of fat replacer has the best effects [1-3]. Several investigations have been suggested the considerable effects of substitution of saturated fat of the meat products with fat replacers such as carrageenan, inulin, guar and xanthan gums [1-4].

Inulin is a soluble plant fiber that consists of a mixture of oligo- and polysaccharides [5]. Inulin can be used as a fat replacement in food products due to its ability to form a gel when mixed with water. The resulting gel has a fine, creamy texture that mimics the oral tactile sensation of fat in products with low fat content [6, 7]. At the same time, inulin contributes few calories to food products, approximately 1 to 1.5 kcal/g [6, 7].

Carrageenans is a sulphated polysaccharides extracted from red seaweed (marine algae of the class Rhodophyta) which can increase viscosity, provide mouthfeel and texturize of the food products [8-10]. Carrageenans, mainly κ and l-carrageenan, due to their ability to combine and form double helices and their interaction with meat contents are suitable structure-forming hydrocolloids for meat products [8-10]. Lambda carrageenan does not form gels in aqueous solutions; however, ƛ-carrageenan is able to form gels [8-10]. Heat-resistance properties of inulin and carrageenan make them suitable to use as a fat replacer in heated-meat products like heated-sausage [8-11]. However, there were no previously published data in this field in Iran. Therefore, the present investigation was carried out in order to study the effects of inulin and carrageenan as a fat replacer on physicochemical, textural and sensory properties of heated-sausage.

MATERIALS AND METHODS

Ingredients. Long chain inulin (Sensus, Netherland) and kappa-carrageenan (Sensus, Netherland) due to their heat-resistance properties were used as fat replacers. Meat (bone-free calf and lamb) were obtained from the Meat Processing Unit of the Isfahan city, Iran; the excess of fat content of the partially thawed meat was cut and separated. The meat was sliced into 1 cm^2 pieces before use. Polyphosphates, nitrites, ascorbate, and condiments were also purchased (Sigma, UK).

TABLE 1
Formulation of sausages prepared with inulin and carrageenan.

Ingredients	Amounts (Kg) in 100 kg sausage in various treatments							
	T0	T1	T2	T3	T4	T5	T6	T7
Meat	52.91	52.91	52.91	52.91	52.91	52.91	52.91	52.91
Fat	19.47	16.55	16.55	13.63	16.55	13.63	16.55	13.63
Ice	20.37	20.37	20.37	20.37	20.37	20.37	20.37	20.37
Inulin	0	0	2.92	5.84	1.46	2.92	0	0
Carrageenan	0	0	0	0	1.46	2.92	2.92	5.84
Starch	4.39	4.39	4.39	4.39	4.39	4.39	4.39	4.39
Salt	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Polyphosphates	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Nitrates	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Sodium ascorbate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sausage condiment	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Treatments. Treatments were prepared based on the method described previously by Méndez-Zamora et al. [12] with some modifications. Table 1 represents all treatments of sausage prepared in this study. Eight treatments including T0 (control group), T1 (low fat group), T2 (2.92% inulin), T3 (5.84% inulin), T4 (1.46% inulin and 1.46% carrageenan), T5 (2.92% inulin and 2.92% carrageenan), T6 (5.84% carrageenan) and T7 (2.92% carrageenan) were prepared in this investigation.

Sausage preparation. Ingredients and raw materials were added per kg of product (Table 1). Meat samples partially thawed were chopped using a cutter (Hobart Corporation, USA) for 3 min, and nitrites, ascorbate, and 1/3 of ice were added slowly. Then, polyphosphates and the remaining 1/3 of ice were incorporated, and the milling continued for 2 minutes. In the next stage, the sausage condiment was added, and the process of emulsification continued for 2 more minutes, keeping the temperature below 11 °C. The partially thawed fat was added and milled for 2 min. The starch was then added along with the rest of the ice, and the milling continued for 3 min. Once the meat dough was prepared, it was stuffed into 3 cm diameter cellulose casings using a Torrey mill (M-22 R1; N.L., México) adapted with a mouthpiece, and the sausages were manually tied with a thread every 15 cm. Subsequently, the sausages were cooked in 80 °C for 65 min. Sausages were then cooled in an ice bath (4 °C for 20 min), drained for 10 min, and refrigerated at 4 °C in polyethylene bags until analysis. Inulin and κ-carrageenan powders were added in the third step after the sausage condiment was added.

Moisture content. The moisture content was determined by weighting 5 g of the samples and drying in an oven (SW-90D, Sang Woo Scientific Co., Bucheon, Korea) at 105 °C for 24 h until reaching a constant weight as mentioned in the method of AOAC [13].

Fat content. Fat content was measured by the Soxhlet method with a solvent extraction system (SOXTEC Avanti 2050 Auto System, Foss Tecator AB, Hoganas, Sweden) based on the method of AOAC [14].

Protein content. Total protein content was determined according to Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (Kjeltec 2300 Analyzer Unit, Foss Analytical AB, Hoganas, Sweden), which is used to determine the amount of nitrogen (%) and to calculate the ratio of protein by multiplying the amount of nitrogen to the constant factor (6.25) as mentioned in the method of AOAC [15].

Ash content. Five gram of each sample was put inside a muffle furnace at 550°C as mentioned in the method of AOAC [16].

Color analysis. Color was measured directly in the external parts of fried sausages using a HunterLab colorimeter (Brookfield, USA) and L*, a* and b* parameters were analyzed. Device was calibrated before measurements. These were performed in triplicate in sections of 2.5 cm long and 3 cm diameter.

Sensory analysis. Sensory evaluation (color, hardness, odor, taste, appearance and overall acceptability) was carried out for all the inoculated and control sausage samples using 10 trained panelists. For each sample, a score sheet from 1-9; 1 represents very dislike while 9 is very like were used. Sensory evaluation was done on fried sausage samples.

Statistical analysis. All tests were done 3 times. SPSS software (ver.18.0) was used to statistical analysis. The obtained data were analyzed by one-way (ANOVA), and significant differences ($p \leq 0.05$) among the means were compared using the Duncan' s test.

TABLE 2
Physicochemical properties of eight treatments of sausage.

Treatments	Physicochemical properties			
	Moisture	Fat	Protein	Ash
T0	57.62±2.27b	17.44±1.33a	10.39±0.46b	5.47±0.19a
T1	61.71±4.36a	8.83±0.48b	10.32±0.71b	5.40±0.37a
T2	59.18±3.69a	7.27±0.54b	11.38±0.84a	5.58±0.40a
T3	60.76±5.41a	6.21±0.028b	12.04±0.93a	5.71±0.24a
T4	59.25±5.03a	7.91±0.63b	11.06±1.03a	5.53±0.35a
T5	62.71±4.17a	6.33±0.47b	11.53±0.88a	5.67±0.39a
T6	58.64±4.36b	7.42±0.26b	11.35±0.90a	5.52±0.56a
T7	60.33±3.84a	6.37±0.19b	12.0±1.13a	5.63±0.67a

*Dissimilar letters in each column showed a significant difference in the level of 5% error.

TABLE 3
Color properties of eight treatments of sausage.

Treatments	Physicochemical properties		
	L*	a*	b*
T0	64.68±4.32a*	9.11±0.77c	11.36±0.92b
T1	63.08±5.14a	9.98±0.54a	11.35±0.87b
T2	62.61±4.49a	9.86±0.80a	11.38±1.00b
T3	57.76±4.34b	9.85±0.62a	11.40±0.66b
T4	62.05±3.68a	9.64±0.41b	11.42±1.05b
T5	61.88±5.27a	9.63±0.71b	11.53±0.94a
T6	60.31±5.71a	9.84±0.61a	11.55±1.01a
T7	55.22±3.20c	9.82±0.27a	11.60±0.99a

*Dissimilar letters in each column showed a significant difference in the level of 5% error.

TABLE 4
Sensory properties of eight treatments of sausage.

Treatments	Physicochemical properties					
	Color	Appearance	Odor	Hardness	Taste	overall acceptability
T0	5.40±5.11b*	5.41±5.33a	5.30±4.41a	5.45±3.14a	5.33±4.15a	5.46±4.85a
T1	5.52±4.32b	5.42±4.72a	4.73±4.62c	5.30±5.07b	5.10±5.20a	5.00±5.11b
T2	5.69±3.68a	5.40±4.61a	5.13±3.98b	5.39±4.44b	5.21±4.46a	5.33±4.23b
T3	5.30±4.21b	5.43±3.28a	5.24±4.06a	5.52±4.10a	5.30±3.21a	5.42±3.17b
T4	5.36±4.27b	5.41±3.22a	5.14±4.10b	5.34±4.07b	5.25±4.52b	5.35±3.82b
T5	5.70±3.36a	5.44±4.35a	5.30±3.11a	5.56±4.28a	5.39±2.71a	5.51±3.93a
T6	5.67±5.19a	5.42±5.61a	5.11±4.06b	5.41±5.11a	5.17±4.29a	5.35±4.10b
T7	5.29±4.25c	5.40±4.28a	5.20±4.18a	5.49±4.92a	5.22±3.13b	5.45±3.98a

*Dissimilar letters in each column showed a significant difference in the level of 5% error

RESULTS AND DISCUSSION

Physicochemical properties of sausage treatments. Table 2 represents the physicochemical properties of all sausage treatments. Results showed that the percent of moisture, fat, protein and ash had considerable changes with reduce in the levels of fat. We found that fat reduction caused enhancement in the percent of moisture, while caused decrease in the levels of protein, fat and ash content of sausage. In the other hand, increase in the levels of inulin and carrageenan caused increase in the percent of moisture, protein and ash and decrease in the percent of fat ($P< 0.05$). The highest percent of moisture, fat, protein and ash was found in the T5, T0, T3 and finally T3 treatments. The lowest percent of moisture, fat, protein and ash was found in the T0, T3, T1 and finally T1 treatments.

Color properties of sausage samples. Table 3 represents the color properties of various treatments of sausage samples. We found that reduction of fat from the formulation of sausage samples caused decrease in the lightness (L*) and also b* parameter, while increase in the redness (a*). Results showed that increase in the levels of inulin and carrageenan caused decrease in the levels of L* and a*, while caused increase in the levels of b* parameter ($P< 0.05$).

Sensory properties of prepared sausage samples. Table 4 represent the sensory properties of prepared sausage samples. We found that increase in the levels of inulin and carrageenan caused increase in the mean scores of judges to all parameters excluding appearance which had no significant changes. The mean scores for the odor parameter in the control group was entirely higher than others.

The highest scores to the color, appearance, odor, hardness, taste and overall acceptability was for T5 treatment of sausage ($P< 0.05$).

Improve in the physicochemical properties of heated-sausage is the first finding of our study due to the replacement of fat in the formulation with inulin and carrageenan. Fat, moisture, protein and ash are the most important characters of the food staffs. It is because of they have considerable effects on the taste, odor, texture, color and also acceptability of food products. The level of protein especially in sausage is considered to be an indicator for its nutritional value. Presence of fats helps to better frying of food products and also give it better taste. However, high presence of fats and especially saturated fats in the food products has harmful effects on the human health. High levels of moisture increase the risk of spoilage and also makes bad changes on the texture of products.

We found that reduce in the levels of fat in formulation of sausage cause increase in the percent of moisture. This finding is maybe due to the effect of fat on the retention of moisture in the sausage samples. By reducing the amount of fat, lesser amount of moisture in its absorbed shape remains in the sample and thus the moisture will increase. Decrease in the levels of fat caused decrease in the levels of protein in sausage but its change wasn't significant. There was no logical reason for this finding but it may be due to the interactions of the fat and protein in the sausage formulation. Fats have no significant effects on the levels of ash of the food products. Therefore, by reducing the amount of fat in the sausage formulation, the levels of ash will not change significantly. We also found that inulin and carrageenan caused increase in the levels of moisture. It is because of inulin and carrageenan absorb the moisture of environment and caused to increase in the levels of moisture in sausage formulation. However, this increase in the levels of moisture is not significantly high and cannot cause bacterial spoilage of sausage. In the other hand, this increase is not related to the increase in the levels of activated water (AW) which is available for bacteria. Šojić et al. [17] showed that the percent of moisture in the control sample was 57.55% and the percent of moisture in samples treated with 5% inulin was 63.67% which was similar to our findings. Huang et al. [18] showed that increase in the levels of inulin caused decrease in the percent of moisture which was dissimilar to our findings. It is may be due to the different formulation of sausage of their study with us and also type and method of using of inulin. Similar reports were found for the effects of carrageenan [8-10].

Increase in the levels of inulin and carrageenan caused decrease in the percent of fat in sausage. It is because of decrease in the amount of fat in formulation of sausage and also its replacement with inulin and carrageenan. Decrease in the levels of fat in sausage and also decrease in the amount of released energy from the fat burning in body make this kind of sausage as dietary product. Menegas et al. [19] reported that adding of inulin caused decrease in the levels of fat (24.4%) compared to the control (45.4%) and also the control group with 50% of fat (28.2%). Similar findings were reported by Méndez-Zamdra et al. [12], Šojić et al. [17] and Huang et al. [18]. Totosaus et al. [20] showed that replacement of fat in the formulation of sausage with κ-carrageenan caused significant reduce in the levels of fat and sodium chloride which was similar to our results.

We also found that inulin and carrageenan substitution caused increase in the percent of protein in sausage samples. The reason for this finding is may be due to the effects of fat reduction on the protein in sausage formulation. Fat reduction in the sausage emulsion caused aggregation of the hydrophilic proteins which may cause increase in the levels of protein in sausage samples. Savic [21] reported that the samples of chicken sausage which were produced using 1%, 2% and 3% inulin had the higher levels of protein compare to the control group. They showed that sausage samples with 3% inulin had the highest percent of protein which was similar to our results. Mendoza et al. [22] reported that the percent of protein in control group and also treatments of 7.5%, 12%, 12.50% and 14% inulin were 24%, 33.50%, 34.10%, 27.30% and 27.60%, respectively which showed increase in the levels of protein up to the 12% concentration of inulin in sausage formulation.

Increase in the substitution of inulin and carrageenan cause increase in the percent of ash in sausage which was due to the effects of inulin and carrageenan. In fact, using from long chain inulin and κ-carrageenan due to their high resistance against heating is the main factor for higher percent of ash in sausage treated with inulin and carrageenan. Similar results were reported by Méndez-Zamdra et al. [12], Šojić et al. [17], Huang et al. [18] and Totosaus et al. [20].

Color properties are another important finding of our study. Results showed that decrease in the levels of fat caused decrease in the lightness of sausage samples. This is mainly due to the application of white fat in the formulation of sausage and therefore its removal makes the appearance of sausage dark which caused reduction of L* parameter. Decrease in the levels of fat caused increase in the levels of redness (a*) and decrease in the levels of yellowness (b*). Addition of inulin and carrageenan into the formulation of sausage caused decrease in the levels of L* factor. It is because of removing of white fat from the formulation. However, this darkness and decrease of the lightness of the sausage samples were not at the levels which cause reducing of marketability of products. Decrease of the lightness in application of carrageenan was entirely higher than inulin which may be due to the lighter color of the inulin powder used in the sausage formulation. Addition of

inulin and carrageenan into the formulation of sausage caused decrease in the levels of a* factor. Besides, addition of inulin and carrageenan into the formulation of sausage caused increase in the levels of b* factor which was higher in samples treated with carrageenan. It is because of carrageenan powder has a yellow color. Šojić et al. [17] showed that the amount of L*, a* and b* factors in the control and treatment samples of sausage were 69.74, 66.21 and 12.08 and 16.18, 14.18 and 13.39, respectively which was similar to our findings. The main reason for the higher levels of redness in treatment with high amount of inulin and carrageenan and low amount of fat is due to the redness of meat used for preparation of sausage. Jiménez-Colmenero et al. [23] reported that changes in the levels of meat and fat and also addition of edible fibers are the main factors for change in the color factors. Sensory evaluation of the sausage samples confirmed some of our findings achieved from physicochemical and color-based tests. We found that the highest scores were gave to the sausage group treated with of 2.92% inulin and 2.92% carrageenan. Addition of inulin and carrageenan into the formulation of sausage caused increase in the scores gave to all parameters. Removal of fat from the formulation caused decrease in the hardness of sausage and then addition of inulin and carrageenan caused increase in the levels of hardness. Findings of previous studies [12, 17, 18, 20] showed that gums had the highest effects on the texture and color of low fat sausages. They showed that in the high concentration of inulin and carrageenan scores become increased even higher than the control group which was considerable.

CONCLUSION

In conclusion, increase of inulin and carrageenan at 2.92% replacement had the highest physicochemical, color and sensory results. This formulation can be a good approach for Iranian factories to producing weight loss heated sausage. Better taste, texture, color and lower price of sausage are another factors which can support the production of this types of sausage.

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A SURVEY ON SELECTED CHEMICAL, MINERAL AND HEAVY METAL PROPERTIES OF MULBERRY PEKMEZ A TRADITIONAL TURKISH PRODUCT

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ABSTRACT

Mulberry pekmez, a traditional Turkish mulberry product, is commonly made around Ordu and other cities in Black-sea region of Turkey. In this study, determined some properties of the mulberry pekmez produced in the Ordu province in Turkey. Mulberry pekmez has a range of dry matter 72.4±1.13-79.2±0.92 %, acidity 136±1.6-206±8.5 meq/kg, pH 5.05±0.07-5.42±0.07, ash content 2.14±0.08-3.40±0.12, fructose/glucose ratio 1.05±0.2-1.20±0.2, %, total sucrose 5.80±0.3-13.10±0.7% and hydroxyl-methyl-furfural (HMF) content 31.9±3.1-540.0±7.9 mg/kg. The mulbery pekmez is a high source of minerals such as; Mn (1486 mg/100g), Ca (109.7 mg/100g), Na (17.09 mg/100g), Mg (18.72 mg/100g), Fe (31.03 mg/100g), Zn (15.44 mg/100g) and Cu (22.42 mg/100g). Other minerals; Cr, Cd, Co, Pb, Sn and Ni as in pekmez is found trace mount. As a result, control of the mulberry pekmez produced in Ordu province should be analyzed more usually than before from farm to table both for the protection of public health and consumer rights.

KEYWORDS:
Mulberry pekmez, chemical, mineral content

INTRODUCTION

Pekmez is one of the most popular and traditional food products, and it is consumed generally for breakfast in Turkey. Pekmez is commonly produced from sugar-rich fruits such as grape and mulberry by concentration of juices up to 70–80 soluble dry matter content. On the other hand, it is produced from sugar beet, sugarcane, sweet sorghum and carob, apple, plum, watermelon, apricot, and fig [1]. Pekmez contains high amounts of sugar, minerals, and organic acids; therefore, it is a very important for food product in human nutrition. Pekmez is a traditional Turkish food made by using different fruits such as grape, mulberry, fig, plum, apple, and harnup, and it is named after the fruit from which it is obtained (i.e., grape pekmez, mulberry pekmez). However, the grape and mulberry are the most common fruits used in processing pekmez in Turkey. Pekmez contains copper, and zinc at definite levels especially it is very rich with iron. In fact, the production of pekmez has been continuously and rapidly increasing because of its composition [2, 3].

The 5-Hydroxymethylfurfural (5-HMF) is a major by-product of thermal processing and results from overheating during sterilization. Upon storage it is formed by hexose dehydration especially at pH 5 or lower, or by the Maillard reaction. Reports have shown 5-HMF to be mutagenic. Therefore, 5-HMF concentration can be used a quality parameter for concentrated food products because high concentrations of 5-HMF are not desirable in pekmez. It indicates the degree of heating of the treated products in various foods containing carbohydrates such as fruit juice and pekmez. The presence of this compound indicates the effects of overheating of the product during manufacture and storage on the quality of pekmez [4].

Mulberry pekmez is highly recommended for colds, stomach complaints, and anaemia. Therefore, the price of mulberry pekmez is higher than the other types. Fresh or dried mulberry is used as a raw material to produce mulberry pekmez. White mulberry is widely grown throughout Turkey. Mulberry fruit is a good source of antioxidant and minerals. The fruit are also rich in sugar, organic acid, and tannin. It is well known in Turkish folk medicine and has been used antimicrobial, antihelminitic drug, anti-inflammatory, and constipation effects. Pekmez has been produced since ancient time but its production technique has not been developed and mechanized. Therefore, the purpose of the study is to determine some chemical, physical, mineral and heavy metal of mulberry pekmez produced in the Ordu province in Turkey.

MATERIALS AND METHODS

Mulberry is an important crop in Turkey and has been cultivated for centuries. About 2.3 million mulberry trees are present in Turkey and approximately 65.000 tons of mulberry fruit are produced annually. The main mulberry harvesting period is between June and August. Pekmez is produced from a variety of fruits or mulberry juices which are boiled without the addition of sugar or other food additives. Mulberry pekmez is commonly produced in small family businesses for their needs and also by small processing plants for commercial purposes. Mulberry pekmez were 10 amounts obtained from different area Ordu provenance of Turkey.

Physicochemical Analyses. Dry matter content was determined by oven-drying 5 g mulberry pekmez at 105°C until a constant weight was obtained [AOAC, 2000]. Process of ashing, cooling and weighing was repeated till no further loss in weight was indicated. The acidity and pH values were determined according to Cemeroglu [5] and pH was measured with a pH meter (OHAUS, USA). Total sugar, fructose/glucose (%) and sucrose were quantities by the Lane-Eynon method [5]. Hydroxy-methylfurfural (HMF) was determined by high performance liquid chromatography [5]. Sucrose determination was done by the titration as methylene blue indicator against reduced sugar solution. Founded total sugar percentage was reduced within the invert sugar percentage and then multiplied by 0.95 rotating factor. The study was designed according to randomized design using 10 pekmez samples. Square root transformation was applied to the apricot mulberry pekmez on each, physicochemical parameter of the mulberry pekmez were estimated using the SSPS® (2000) statistical software.

Mineral and heavy metal analysis. In order to determine the mineral and heavy metal contents of the samples, 2 g of each treatment samples were ashed in a porcelain crucible, solubilized with 10 ml of 6 N HCl, quantitatively transferred into 50 ml volumetric flasks, and diluted to volume with double-deionized water and filtered after 5-6 hours with blue-band filter paper and again regulated to 50 ml [AOAC, 2000]. Concentrations of Calcium (Ca), sodium (Na), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Molybdenum (Mo), Cadmium (Cd), Cobalt (Co), Stannum (Sn), Chromium, (Cr), Nickel (Ni) and Lead (Pb) were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP–OES, Varian Vista-Pro, Australia). All the analyses were performed in duplicate and the results reported as mean values.

Statistical analysis. The study was designed according to randomized design using 10 mulberry pekmez samples factorial experiments. Square root transformation was applied to the pekmez samples on each chemical, mineral and heavy metal content parameter of the samples were estimated by ANOVA using the SSPS® (2000) statistical software. Duncan's Multiple Range Test was used for the determination of statistically different groups.

RESULTS AND DISCUSSION

Physicochemical properties of mulberry pekmez samples. The chemical and physical characteristics of mulberry pekmez are given in Table 1. As shown in Table, mulberry pekmez contained high amounts of total sugar, which is composed of approximately as glucose and fructose. This is very important for human nutrition because of its easy digestibility and also these sugars provide a readily available energy source since they easily pass to blood. In addition, glucose, energy source of the brain, enhances the transport of tryptophan through the blood–brain barrier and it is useful in serotonin synthesis that has a function in brain working [6]. However, this pekmez contains very small contents of sucrose and protein.

Mulberry pekmez is obviously rich with sugar; therefore, it is a good source of energy. Because of high contents of minerals and sugar content, pekmez is very important for human nutrition, especially for babies, children, sportsmen, and pregnant women [3].

The total free acidity shows a mean value of 176.73±24.15 meq/kg with a range between 136 and 206 meq/kg for mulberry pekmez, which is next to what is this value, represents the organic acids content in honey. Free acidity shows a mean

TABLE 1
Chemical and physical characteristics of mulberry pekmez

Properties	Minimum	Maximum	Means±Sd
Dry matter (%)	72.4±1.13	79.2±0.92	76.53±2.29
Acidity (meq/kg)	136±1.60	206±8.5	176.73±24.15
pH	5.05±0.07	5.42±0.07	5.22±0.15
Ash (%)	2.14±0.08	3.40±0.12	2.64±0.53
Fructose/glucose (%)	1.05±0.2	1.20±0.2	1.05±0.05
Total Sucrose (%)	5.80±0.3	13.10±0.7	9.8±2.50
HMF (mg/kg)	31.9±3.1	540.0±7.9	288.28±178.5

HMF: Hydroxy-methylfurfural

TABLE 2
Mineral and heavy metal contents of the mulberry pekmez samples (mg/kg)

Mineral	Minimum	Maximum	Means±Sd
Mn	1205.12±16.92	1685.09±22.04	1486.73±27.36
Ca	87.84±7.23	126.73±14.36	109.27±17.51
Na	15.52±6.35	24.37±4.27	17.09±6.12
Mg	12.48±8.26	29.24±4.80	18.72±5.73
Fe	23.09±3.86	44.22±5.78	31.03±2.75
Zn	12.58±4.28	19.86±5.73	15.44±3.59
Cu	13.51±3.78	24.82±3.86	22.42±3.08
Cr	1.17±0.75	1.49±0.53	1.34±0.43
Cd	0.9±0.04	1.39±0.37	1.13±0.28
Co	0.79±0.07	1.23±0.15	0.89±0.27
Pb	0.09±0.05	0.12±0.12	0.11±0.08
Ni	0.23±0.07	0.27±0.04	0.21±0.10
Sn	0.12±0.13	0.23±0.20	0.57±0.21

value of 20.78 mg/kg; this value represents the organic acids content in honey. The pH value of the mulberry pekmez changed between 5.05 and 5.42. Akbulut has (1) measured pH value of the mulberry pekmez as 4.88; Sengul et al. [7] determined pH value of the carop pekmez as 5.09. In some samples, this result was observed, while some had lower results due to the composition of the raw material.

The sucrose content of mulberry pekmez mulberry pekmez shows a mean value of 9.80% with a range between 5.80-13.10%, which is next to what is normally accepted. The amount of sucrose in mulberry pekmez differs according to the maturity degree and nectar compound of the pekmez. Fructose/Glucose (F/G) ratio has been recommended to evaluate honey granulation, because glucose is less water soluble than fructose. The proportion of fructose to glucose depends largely on the nectar source (8). The F/G ratio in the investigated mulberry pekmez which is between 1.05 and 1.20 values (Table 1).

HMF, an indicator of quality deterioration, occurs as a result of excessive heating in foods containing carbohydrates [5]. Therefore, high amounts of HMF are not desired in processed fruit juices. The Turkish Standard Institute recommends a maximum HMF concentration of 75 mg for first quality mulberry pekmez. In this study, lowest values 31.9 mg/kg and highest 540 mg/kg were found. HMF is not present in fresh, untreated foods, but rapidly accumulates during the heat treatment or long-term storage in carbohydrate-rich products such as processed fruits [9]. The higher pH stimulates the Maillard reaction in high sugar and protein containing medium [10].

Pekmez easily passes into the blood without digestion because most of its carbohydrate is in the form of monosaccharide like glucose and fructose. This is nutritionally important, especially for babies, children, sportsmen, and in situations demanding urgent energy [7].

Mineral and heavy metal properties of mulberry pekmez samples. The mineral contents of mulberry pekmez are shown in Table 2. Tosun and Ustun [10] studied on mulberry pekmez similar Ca and Mg content than in the present study, but lower Na and Mn content. Çakmakcı and Tosun [11] studied pekmez mulberry pekmez added cornelian cherry and they found lower Ca and Na content Mg contents with the present study. Şimşek and Artık [12] reported that Ca and Mg contents in pekmez of grapes growing in Turkey were higher than that of our findings, but lower Na, Mn contents. It can be explained that all the products had different mineral content, because they have different areas. Generally, minerals from plant sources are less bioavailable than those from animal sources. Cvetković et al. [13] studied on dried fruit mulberry pekmez and they found similar Ca and Na, but lower Fe content than in the present study. Clary et al. [14] found lower Ca contents in dried fruit mulberry pekmez with the present study, but higher Fe content. Haight and Gump [15] reported similar that Ca, Na, Fe content, but higher Mg of red and white grape juice concentrate mulberry pekmez lower than that of our findings. Fe and Cu contents of fruit and vegetable play an important role in the final quality of the products as well as their nutritional and biological role, when certain alternative reactions intervene. The presence of high Fe and Cu concentrations from adventitious contamination are damaging to product quality since in an ionized form, they catalyze oxidation reactions of the lipids with development of unusual flavours.

Table 2 shows Pb, Ni, Sn, Cd and Mo heavy metal contents of the mulberry pekmez difference drying methods. The sources of high levels of Pb and Cd are likely to be the transferred from the tin can and salt used in the brine. Overall, our region was an agricultural region, substantial amounts of artificial fertilizers are used and it was also very dusty. Cd, Co, Pb, and Zn elements may be passed to herbs by means of wind-blown dust, soil and

water. Other inorganic elements which may contribute to biological processes, but which have not been established as essential, are barium, bromine, cadmium, lead and lithium [16]. As a conclusion of this study, it can be said that mulberry fruits are a valuable horticultural product, based on their rich and beneficial nutrient composition. Because of the demonstrated high sugar and mineral content mulbery pekmez is very good source in human nutrition [4]. Certain growing conditions and cultural management techniques, affecting the nutritional value of mulberry pekmez will be the subject of further research projects.

CONCLUSION

To summarize, this searches shows novel results of mulberry pekmez composition. These results make the pekmez in Ordu province of Turkey, a product that offers good quality, and showing good beekeeping practices, which must be standardized to maintain or to improve quality in the future. These results are also very important for commercialization of Ordu province of Turkey mulberry pekmez both in the national and international markets.

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MILK SOMATIC CELL COUNT AND PH AS AN INDICATOR OF UDDER HEALTH STATUS IN HOLSTEIN COWS

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ABSTRACT

This study was carried out to investigate the relationships milk somatic cell count (SCC) and pH in Holstein dairy cattle raised in a private dairy farm in Kirsehir province of Turkey. A total of 466 samples collected from 101 first lactation Holstein cows calved in 2015 year were used. Milk samples were collected monthly from individual cows from 30±15 to 150±15 days of lactation and three seasons (autumn, winter, spring and summer). The SCC data were classified into three classes corresponding to the content of SCC: <100.000, 100.000-500.000 and >500,000 cell/ml. The results showed that the effects of stage of lactation and sampling season on logSCC and pH were statistically important (P<0.01). LogSCC and pH values were increased with advancing stage of lactation. LogSCC was significantly higher during summer while pH was higher in spring. Cows with SCC greater than 500,000 cell/ml had higher pH values. To conclude, milk pH should be considered as a criterion for detection of SCC and mastitis.

KEYWORDS:
Holstein cow, somatic cell count, pH, udder

INTRODUCTION

Mastitis is an inflammation of the mammary gland which is caused by the bacterial pathogens or virus. An inflammatory response is initiated when pathogens enter the mammary gland through the teat canal [1]. Somatic cell count (SCC) in milk increases during infection. Therefore, SCC was used an indicator the presence of infection in mammary gland [2].

High SCC in milk reduces the milk quality and its products. SCC affects flavor and deteriorates the physiochemical properties of milk and its shelf life [3]. SCC is also associated with altered fatty acid composition, protein quality, lactose, ions and mineral concentration, besides higher pH and enzymatic activity of raw milk [4, 5].

The new trend has been to find suitable biochemical indicators to detect mastitis [6]. Besides SCC, other tests like pH are also used for udder health assessment. The pH of milk is influenced by hygienic conditions [7]. Raynal-Ljutovac et al. [8] also reported that SCC is positively related to milk pH. Good quality milk has a pH around 6.5 to 6.7 which is also ideal for the growth of many beneficial microorganisms and this reduces by the development of acidity [9].

Non-sufficient report has been revealed the relationships between SCC and pH. Especially, there has been a few reports about effective factors on SCC and pH in dairy cows. Thus, further studies have been needed to determine on milk SCC and pH. The present study was aimed to determine the relationships between SCC and pH and effective factor on these parameters.

MATERIALS AND METHODS

A total of 466 records from 101 Holstein primiparous cows calved in the years 2015 were used in this study. These data were provided from a private commercial dairy farm in Kirsehir province, Turkey. Milk samples (approximately 50 ml) were collected into sterile tubes from mornings 5 times monthly with regular intervals in the 30±15, 60±15, 90±15, 120±15 and 150±15th day of lactation. These tubes were kept in ice during transport to the laboratory and stored at +4°C for the milk SCC and pH analyses. The milk samples were heated for 15 minutes in the water bath at 37.5°C and consequently cooled to 20 °C by careful stirring.

SCC were measured by using the DeLaval cell counter® (DCC). The pH value was determined by the portable pH meter (Testo 206, Testo Ltd, Alton, Hampshire, UK).

The cows were grouped according to stage of lactation 1 to 5 month and four sampling seasons as winter (December to February; *n=156*), spring (March to May; *n=62*), summer (June to August; *n=86*) and autumn (September to November; *n=162*). The SCC data were divided into three classes corresponding to the content of SCC: <100.000 cell/ml *(n=304)*, 100.000-500.000 cell/ml *(n=136)* and >500,000 cell/ml *(n=26)*.

After SCC values were transformed to $\log_{10}$ for normality and homogeneity of variances, GLM procedure of SPSS 16 statistical software was applied

on obtained data. The significant differences between means were determined by Duncan Multiple Range Test within the same software.

RESULTS AND DISCUSSION

The overall mean SCC and pH values were determined as 157472±16057 cell/ml and 6.52±0.023, respectively. The mean logSCC was determined as 4.85±0.023. Stage of lactation affected on logSCC was significantly important (P<0.01). As seen in Figure 1, the higher logSCC were determined in the 3th and 5th lactation months, but the lowest value in the 1st lactation month. LogSCC increased with the progressive of stage of lactation. This result is agreeing with the finding of Syridion et al. [10], who reported that the milk SCC was higher during late lactation period. However, Erdem et al. [11] found that milk SCC was not affected by stage of lactation. Djabri et al. [12] reported that the SCC tended to be higher in very early and late lactation and at minimum level in mid-lactation. Bartlett et al. [13] stressed that SCC increased in late lactation. This may be due to the increased prevalence of infection and permanent glandular damage from previous infections.

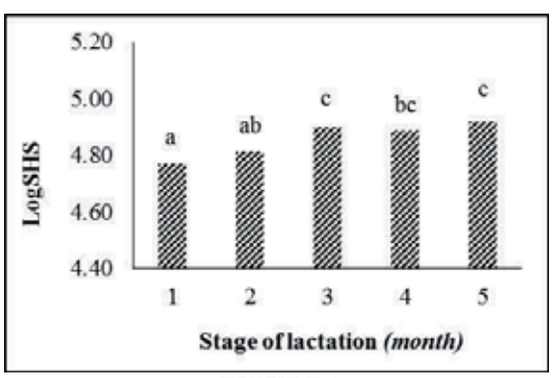

FIGURE 1
Changes in the logSCC according to stages of lactation

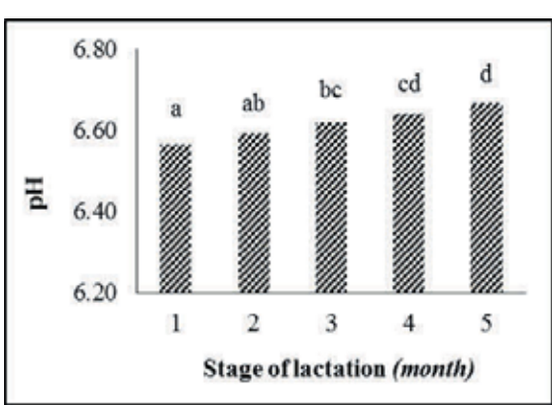

FIGURE 2
Changes in the pH according to stages of lactation

pH was significantly affected by stage of lactation in this study (P<0.01). The highest pH was determined in the 1st, but the lowest value in the 5th lactation month (Figure 2). The results showed that the milk pH increased gradually with advancing stage of lactation. Similarly, Atasever et al. [14] found that the effect of test day on pH was significant (P<0.05). Pavič et al. [7] observed a gradual increase in pH values during lactation. Conversely, Syridion et al. [10] determined that there were no significant differences between milk pH values in different lactation stages. Şekerden and Avşar [15] and Waghmare [16] reported that milk pH reduced with the progress of lactation.

Sampling season had a significant effect on logSCC (Figure 3). The highest logSCC was found in summer, but lowest in autumn. Similar results were obtained by other researches [2, 10], who reported that the SCC values were significantly higher in summer. Erdem et al. [11] and Khate and Yadav [17] found that the SCC was generally highest in summer and lowest in winter season. Singh and Ludri [18] also observed that SCC was highest during the hot-humid season. De Haas et al. [19] stressed that the high temperature in summer was the reason of the increased SCC. This effect may be most likely due to the stress factors on cow caused higher concentration of pathogens such as high temperature [11] and thermal stress [20] during summer season.

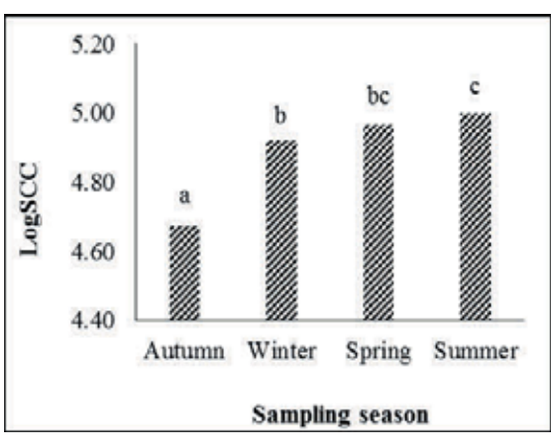

FIGURE 3
Changes in the logSCC according to sampling seasons

Sampling season affected pH values significantly (P<0.01). The highest pH was determined in spring, but lowest in autumn season (Figure 4). This result is different than the findings of Syridion et al. [10], who found that effect of seasons on pH was no significant. Ozrenk and Inci [21] also reported that pH values in winter and summer were not different.

As a seen Figure 5, SCC groups had a significant effect on pH (P<0.01). Milk pH compared to the group with SCC>500.000 cells/ml. Şahin et al. [22] found that effect of SCC on pH was statically important. Raji et al. [23] reported that the increase in

SCC might be attributed to the increased pH. Sena and Sahmani [24], Poulsen *et al.* [25] and Raji et al. [23] found that the significant differences in milk pH were observed between low and medium SCC levels as well as medium and high SCC levels. Bharti et al. [26] determined that the mean pH value for healthy group was lower as compared to subclinical infected group, which is in agreement with the results of previous studies [26, 27, 28]. Ogola et al. [5] reported that the pH of milk in Kenyan Zebu was 6.63±0.03 in healthy quarter and 6.75±0.03 in infected quarter. Conversely, Bansal et al. [29] for Buffalo and Guha and Gera [6] for dairy cows found that pH was not different in cows with normal and infected cows. According to Poulsen et al. [25] the altered pH levels in raw milk from individual cows reflected mastitis, where an impaired barrier between blood and milk raised milk pH toward the higher pH of the blood.

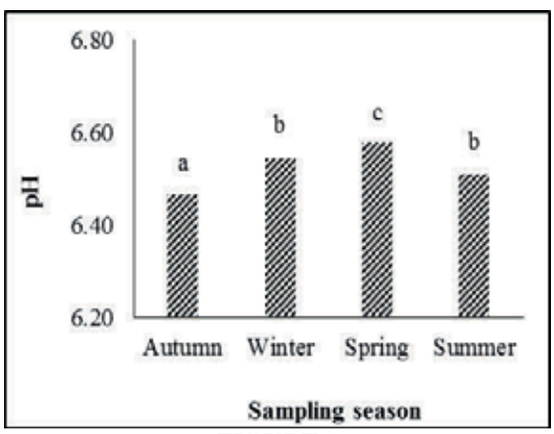

FIGURE 4
Changes of in the pH according to sampling seasons

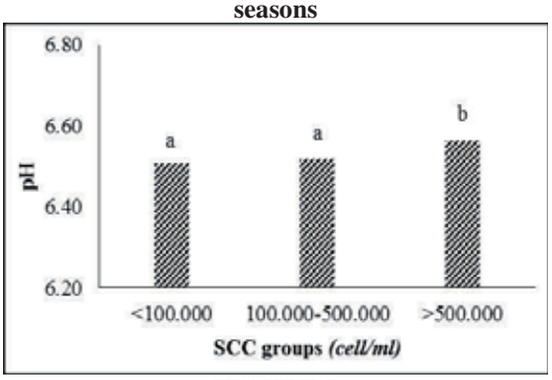

FIGURE 5
Effect of SCC groups on pH

Milk constituents being responsible for pH are casein citrate, phosphate and dissolved carbon dioxide and bicarbonates. In mastitis, the increased permeability of the gland to blood components viz., bicarbonate ions results in higher values of pH in the milk [30]. These changes might be linked to the increased permeability of the mammary epithelium cell lead to the transfer of components from blood to milk such as citrates and bicarbonates that caused the elevated pH levels [31]. These properties may be responsible for normalize the SCC and pH of the milk [16].

CONCLUSIONS

Present study showed that the milk SCC and pH in Holstein cows were significantly influenced by the stage of lactation and sampling season. SCC and pH increased during lactation period and hot seasons. As seen that, these environmental factors to low SCC and pH should be considered carefully. This study also revealed that milk pH was affected by the level of SCC. Especially, the highest pH was determined in particular cows with SCC greater than 500.000 cell/ml. In conclusion, milk pH can be used as a reliable criterion for detection of SCC and mastitis.

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