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Effect of nonionic vs. ionic-based surfactants on molecular morphology and fractionations, and *in situ* mobile nylon bag nutrients disappearance of the steam-infrared heated- flaked corn grains

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Abstract The present experiment evaluated the effect of applying nonionic [(leaves of *Laurus nobilis* (LN) and Tween 80 (TW))] or ionic-based [(sodium dodecyl sulfate (SD) and double sulfate of aluminum and potassium (AL))] surfactants, applied during steaming to steam-infrared heated-flaked corn grains (SIFC), on nutrient composition, crude protein (CP) and carbohydrate fractionations, protein and starch granule structures, and *in situ* mobile nylon bag ruminal and post-ruminal nutrients disappearance. Treatments were steam-infrared heated-flaked corn grain (Control; $SIFC_{CO}$), and SIFC treated with LN ($SIFC_{LN}$), TW ($SIFC_{TW}$), SD ($SIFC_{SD}$) or AL ($SIFC_{AL}$). Surfactants were used at 10 g/kg dry matter (DM). The CP concentration was highest in $SIFC_{CO}$ and lowest in $SIFC_{AL}$ (92.9 and 85.4 g/kg DM, respectively). Indigestible CP was higher in $SIFC_{SD}$ (6.67% CP) than those of the other treatments (P<0.05). Total carbohydrate fractionation was the highest in $SIFC_{AL}$ treatment (P<0.05). Both $SIFC_{AL}$ and $SIFC_{TW}$ (19.5 and 19.7 g/kg DM, respectively) had the highest concentration of soluble fiber compared with the other treatments (P<0.05). The $SIFC_{AL}$ had the greatest concentration of digestible fiber (96.1 g/kg DM). Fourier-transform infrared spectroscopy (FTIR) wave number peaks for amide I and amide II were greater for $SIFC_{CO}$ and $SIFC_{SD}$ than the other treatments. Starch morphology granules were notably changed in $SIFC_{TW}$. When SD was used, both post-rumen and total tract disappearances of DM, CP, and starch were significantly increased (P<0.05) compared to other treatments. The highest ruminal disappearances of CP and starch were seen in $SIFC_{LN}$ and $SIFC_{SD}$, respectively. Overall, the present results demonstrated that either the nonionic-based or ionic-based surfactants might be beneficial when applied during the steam cooking of the corn grains, considering their positive effects on protein starch morphology, as well as digestive disappearance of CP and starch.

Keywords: corn, steam-flaking, protein, starch

Introduction

Starch is a major component of cereal carbohydrates as well as energy source for dairy cows (Ferraretto et al., 2013). Therefore, milk yield efficiency could increase by improvement in starch utilization. Increased starch consumption may gain the production of ruminal volatile fatty acid (VFA) and lactate, as well as obtain a more acidic environment in the rumen (Dijkstra et al., 2012). This is an important point because regulation of rumen fermentation is critical to ensure successful cow health management (Humer et al., 2018). Therefore, processing of cereal grains has been used as a tool to optimize ruminal starch digestibility (Tosta et al., 2019). Steam-faking meliorates starch availability, nutrient utilization, and overall feeding quality (Zinn et al., 2002). Compared to unprocessed corn grain, steam-flaking corn increased starch gelatinization from 16.70 in whole grain to 56.30 g/kg (Qiao et al., 2015). It is generally believed that particle size of steam-flaking corn caused a longer retention time leading to increased starch digestibility (Martins et al., 2019), as well as, providing more energy for rumen microorganisms and increasing the rate of microbial protein supply to the small intestine (Dhiman et al., 2002). Infrared radiation technique in physical grain processing, known as micronization, is also a kind of providing heat and wave inside the grain; this increases molecular movement and causes a rapid rise in the interior temperature (Cheraghi-Kamalan et al., 2019). Micronization of grains increased the starch and protein digestibility in both rumen and small intestine, and reduced starch availability to the large intestine while decreasing the hindgut acidosis (Sajjadi et al., 2022). As a novel type of feed additive used in ruminant nutrition, surfactants (chemical surface-active agents and biosurfactants) effectively enhance the quality of processed grains, and boost productivity by increasing the emulsification of ruminal liquid, increasing the amount of ruminal microorganisms, stimulating the endogenous microbial enzymes, and changing the levels and proportion of VFAs (Liu et al., 2013). Therefore, we hypothesized that adding surfactants during steam-flaking of corn grains may modify *in situ* mobile nylon bag disappearance of the nutrients. Besides, processing with surfactants may have a potential to improve the morphology and structure of the molecules. Thus, the objective of the present study was to evaluate the physically-processed corn grains, using steam-infrared heated-flaking for their effects on the nutritional value, crude protein (CP) and fiber fractions, as well as *in situ* mobile nylon bag nutrient digestibility, and molecular structures.

Materials and methods

The animal study was performed according to protocol Institutional Animal Care Committee, Ferdowsi University of Mashhad (Protocol number 101984).

Corn grain processing

The nutrient composition of intact corn grain was: CP= 88.9, neutral detergent fiber (NDF)= 101.9, acid detergent fiber (ADF)= 43.7 and Starch= 702.3 g/kg (dry matter) DM. Experimental treatments were: steam-infrared heated- flaked corn grains (control; $SIFC_{CO}$), and $SIFC_{CO}$ treated with leaves of *Laurus nobilis* ($SIFC_{LN}$), Tween 80 ($SIFC_{TW}$), sodium dodecyl sulfate ($SIFC_{SD}$), or double sulfates of aluminum and potassium ($SIFC_{AL}$). The chemicals and organic surfactants were applied during the steaming at 10 g/kg DM of corn grains. Surfactants were mixed with the grains and steamed for 35 min at 96 °C. Steam cooked grains were infrared-heated for 55 seconds to reach the internal kernel temperatures of 100°C, and then flaked using a roller mill in a flaker machine.

Nutrient composition, carbohydrate and protein fractionations

Samples were dried for 48 h at 60 °C employing an air-forced oven to calculate the DM content. Then, milled and passed a 2 mm screen for chemical analyses. Concentrations of ether extract (EE) and ash were determined, as explained in AOAC (2012). The NDF and ADF contents were measured (Van Soest et al., 1991) without sodium sulfite and heat-stable alpha-amylase, and the results were reported without residual ash. Nitrogen content was measured by Kjeldahl procedure (Kjeltec 2300 Autoanalyzer by Foss Tecator AB in Hoganas, Sweden), and CP concentration was calculated as N×6.25. The starch content was determined utilizing the anthrone and sulfuric acid method (Rose et al., 1991). The water-soluble carbohydrate (WSC) fraction was quantified using phenol/sulfuric acid (Hall, 2014). The amylose and amylopectin contents were determined according to Hu et al. (2010). The CP was partitioned into five fractions including ammonia (A_1), soluble protein (A_2), insoluble true protein (B_1), fiber-bound protein (B_2) and indigestible protein (C). The quantity of ammonia was zero in samples (Van Amburgh et al., 2015). Carbohydrate fractions were measured as water soluble carbohydrates (CA_4), starch (CB_1), soluble fiber (CB_2), digestible fiber (CB_3), and ingestible fiber (CC), according to (Van Amburgh et al., 2015).

Fourier-transform infrared spectroscopy (FTIR)

The infrared (IR) absorbance band of the treatments were created at the Department of Chemistry, Ferdowsi University Mashhad, Iran, using FTIR spectroscopy (Thermo-Nicolet AVATAR 370 FT-IR, Avatar 370, Thermo Nicolet Corporation, America), with a FTIR spectral range of 400-4000 cm^{-1}. Molecular spectral regions related to protein molecular structures were recorded. The primary protein structures were: amide I (1,720 to 1,577 cm^{-1}) and amide II (1,577 to 1,486 cm^{-1}). The relative contribution of the α-helix (1,650 cm^{-1}) and β-sheet (1,638 cm^{-1}) protein secondary structure on the amide I absorption band was determined using the second derivative spectral peaks (Yu, 2010).

Scanning electron microscopy

Samples frozen in liquid nitrogen were processed into sub-sections to ensure optimal scanning quality, covered with 10 Å gold palladium in a Hummer sputter coating, and then images were prepared (Scanning electron microscope, LEO 1450 VP, USA). The samples were scanned using an accelerating voltage of 25 kV at 2500 × magnification.

X-ray diffraction measurements (XRD)

The X-ray diffraction analyses were run at a Cu-Kα, using radiation wavelength of 1.54 Ā, a target voltage of 45 kV, current of 44 mA, and diffraction angular range of 5-40° (2θ) and step size of 0.02 (XMP300, Unisantis, Georgsmarienhutte, Germany). Interpretation of XRD results was done by the Match software version 3.15.

Rumen in situ mobile bag technique

Ruminal, post-ruminal and total tract DM, CP and starch disappearances were measured using *in situ* mobile nylon bag techniques (Mesgaran and Stern, 2005; Kheirandish et al., 2022). Briefly, three lactating Holstein cows, fitted with ruminal cannulas (645± 17 kg body weight, 170±11 days in milk), were fed a diet containing a forage:concentrate ratio of 45:55. The forages consisted of alfalfa hay and corn silage, 300 and 700 g/kg DM, respectively. The concentrates contained barley grain, corn grain, soybean meal, cottonseed meal, wheat bran, fish meal, fat powder, sodium carbonate, calcium carbonate, mineral and vitamin premix and salt, at 250, 250, 200, 120, 100, 30, 20, 10, 5, 10 and 5 g/kg DM, respectively. Approximately, 6 g of each grounded sample were put into a polyester bag (12×17 cm, 50 µm pore size, n=6) and transferred to the rumen just before the morning meal. The bags were removed after 12 h, washed using a washing machine, dried at 60 ˚C for 48 h using air-forced oven drier, weighed and stored for determination of DM, and CP, and starch disappearances (the difference between the primary sample and the residual after incubation in the rumen). Approximately, 1 g of the residuals in each bag was transferred into a mobile nylon bag (3×6 cm; 52 µm pore size; 6 bags per each sample). The bags were inserted into duodenum through a T-shaped cannula at the rate of one bag every 30 minutes. The bags were removed from the evacuated feces, laundered in the washing machine until the water remained clear, and then dried (60°C, 48 h). Finally, the bags were weighed to calculate DM, and analyzed for CP and starch content. The difference between the rumen incubated residual and the part remaining in the samples recovered from feces, represented the post-rumen disappearance.

Statistical analysis

Data on nutrient composition, CP and carbohydrate fractions were determined in 4 replications. Multivariate data analysis of the amide spectral region (1,720-1,480 cm^{-1}) was done to demonstrate differences in the molecular structure between the treatments using software R version 3,3,1. Data were analyzed as a completely randomized design using PROC GLM (SAS, 2004), according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$ where Y: analyzed variable, µ: overall mean for the variable, T_i: effect of the corn processing and e_{ij}: random error associated with the observation i. The Tukey's multiple comparison test was utilized to compare the differences among least square means. Differences were declared significant at P<0.05, and differences at 0.05 < P < 0.10 were expressed as tendency. Orthogonal contrasts were generated to compare 1) $SIFC_{CO}$ vs. $SIFC_{LN}$, $SIFC_{TW}$, SIFCSD and $SIFC_{AL}$, 2) $SIFC_{LN}$ vs. $SIFC_{TW}$ and 3) $SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{TW}$.

Results

Nutrient composition

The concentrations of CP and WSC were significantly varied when corn grains were processed with different surfactants (Table 1; P<0.05). The highest CP content was measured in $SIFC_{CO}$. The $SIFC_{LN}$ had greater CP content than $SIFC_{TW}$ (enhanced by 2.22 %, P= 0.063). The orthogonal contrasts indicated that both $SIFC_{SD}$ and $SIFC_{AL}$ had lower CP concentration compared with $SIFC_{TW}$ (87.5 vs. 90.2 g/kg DM). The $SIFC_{SD}$ (46.5 g/kg DM), exhibited a greater WSC content compared with other treatments. Processing of corn grains with SD and AL resulted in a significant rise (6.85%) in WSC content compared with that processed with TW.

Table 1. The effect of nonionic and ionic-based surfactants on nutrient composition of steam-infrared heated flakes of corn grains

Parameters	Treatments					SEM[6]	P-value			
	Nonionic surfactants			Ionic surfactants			Treatment	Contrasts		
	$SIFC_{CO}$[1]	$SIFC_{LN}$[2]	$SIFC_{TW}$[3]	$SIFC_{SD}$[4]	$SIFC_{AL}$[5]			C[7]	C[8]	C[9]
Ether extract (g/kg)	37.3	37.9	36.9	37.3	37.6	0.695	0.235	0.914	0.521	0.634
Ash (g/kg)	11.6	11.5	11.8	12.2	11.5	0.459	0.432	0.976	0.512	0.511
NDF[10] (g/kg)	99.7	99.9	100.2	99.9	101.8	0.404	0.212	0.721	0.805	0.577
ADF[11] (g/kg)	41.8	41.6	41.6	42.3	42.8	0.680	0.450	0.318	0.487	0.417
Crude protein (g/kg)	92.9[a]	92.2[ab]	90.2[bc]	89.5[c]	85.4[d]	1.34	0.019	0.166	0.063	0.049
Starch (g/kg)	704.5	703.6	702.4	702.9	703.3	1.56	0.984	0.284	0.106	0.159
WSC[12] (g/kg)	44.5[ab]	43.2[b]	42.3[b]	46.5[a]	43.9[ab]	1.41	0.047	0.103	0.141	0.032
Amylose (% of starch)	27.3	27.2	27.2	26.9	27.2	0.219	0.313	0.276	0.671	0.675
Amylopectin (% of starch)	72.7	72.8	72.8	73.1	72.8	0.219	0.313	0.276	0.671	0.675

[1]$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, [2]$SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, [3]$SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, [4]$SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, [5]$SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.
[6]SEM: Standard error of the mean.
Contrasts: [7]$SIFC_{CO}$ vs. $SIFC_{LN}$, $SIFC_{TW}$, SIFCSD and $SIFC_{AL}$, [8] $SIFC_{LN}$ vs. $SIFC_{TW}$, [9] $SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{TW}$.
[10]Neutral detergent fiber, [11]Acid detergent fiber, [12]Water-soluble carbohydrates.
[a,b,c] Within rows, means with common superscript (s) are not different (P>0.05).

Carbohydrate and protein fractions

The results of CP and carbohydrate fractionations of corn grain samples are indicated in Tables 2 and 3, respectively. A significant effect of treatments on C fraction concentration was observed. The highest fraction of C was found for the $SIFC_{SD}$ and the lowest level in $SIFC_{CO}$. The total carbohydrates, CA_4, B_2, B_3 and CC fractions were affected by surfactant treatment ($P<0.05$). The total carbohydrates, B2 and B3 fractions, and also CC fractions were higher in $SIFC_{AL}$ compared with other treatments. The B2 fraction was reduced in $SIFC_{CO}$ compared with other treatments (12.9 vs. 17.87 g/kg DM).

Table 2. The effect of nonionic and ionic-based surfactants on protein fractionation (CNCPS- v 6.5) of steam-infrared heated flakes of corn grains

Parameters	Treatment					[6]SEM	P-value			
		Nonionic surfactants		Ionic surfactants			Treatment	Contrasts		
	[1]$SIFC_{CO}$	[2]$SIFC_{LN}$	[3]$SIFC_{TW}$	[4]$SIFC_{SD}$	[5]$SIFC_{AL}$			[7]1	[8]2	[9]3
Ammonia[10] (A1, % of CP)	0	0	0	0	0	0	0	0	0	0
Soluble true protein[11] (A2, % of CP)	2.13	3.77	3.31	3.40	3.0	1.13	0.394	0.219	0.117	0.182
Insoluble true protein[12] (B1, % of CP)	89.33	87.37	87.64	86.92	88.24	1.24	0.100	0.119	0.238	0.111
Fiber-bound protein[13] (B2, % of CP)	3.13	2.90	2.95	2.99	3.13	0.425	0.527	0.433	0.201	0.104
Indigestible protein[14] (C, % of CP)	5.39[c]	5.95[b]	6.08[b]	6.67[a]	5.60[c]	0.152	0<0.0001	0.104	0.098	0.067

[1]$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, [2]$SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, [3]$SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, [4]$SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, [5]$SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.
[6]SEM: Standard error of the mean.
Contrasts: [7] $SIFC_{CO}$ vs. $SIFC_{LN}$, $SIFC_{TW,}$ SIFCSD and $SIFC_{AL}$, [8] $SIFC_{LN}$ vs. $SIFC_{TW}$, [9] $SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{TW}$
[10]$N\text{-}NH_3 \times 6.25$, [11] non-protein-nitrogen% ((NPN)×6.25) - (%A1/%CP×100), [12]100-%A_2- %B_2- %C, [13](%Neutral detergent-insoluble crude protein (NDIP)-%Acid detergent-insoluble crude protein (ADIP))/%CP ×100, [14] %ADIP/%CP ×100.
[a,b,c] Within rows, means with common superscript (s) are not different ($P>0.05$).

Table 3. The effect of nonionic and ionic-based surfactants on carbohydrate fractionation (CNCPS- v 6.5) of steam-infrared heated flakes of corn grain

Parameters	Treatment					SEM[6]	P-value			
	$SIFC_{CO}$[1]	Nonionic surfactants		Ionic surfactants			Treatment	Contrasts		
		$SIFC_{LN}$[2]	$SIFC_{TW}$[3]	$SIFC_{SD}$[4]	$SIFC_{AL}$[5]			C[7]	C[8]	C[9]
Starch (CB1, g/kg)	704.5	703.6	702.4	702.9	703.3	1.56	0.984	0.284	0.106	0.159
Water soluble carbohydrate (CA4, g/kg))	44.5[ab]	43.2[b]	42.3[b]	46.5[a]	43.9[ab]	1.41	0.047	0.209	0.141	0.094
Total carbohydrates[10] (g/kg)	858[b]	858.3[b]	860.9[b]	861.2[b]	865.3[a]	1.90	0.049	0.271	0.117	0.088
Non fiber carbohydrate[11] (NFC, g/kg))	761.9	761.7	764.2	764.4	764.3	1.95	0.098	0.101	0.236	0.194
Soluble fiber[12] (CB2, g/kg))	12.9[b]	14.9[ab]	19.5[a]	17.4[ab]	19.7[a]	3.07	0.039	0.007	0.0866	0.109
Digestible fiber[13] (CB3, g/kg))	93.9[c]	94.3[bc]	94.5[bc]	94.9[b]	96.1[a]	0.389	0.002	0.065	0.389	0.192
Indigestible fiber[14] (CC, g/kg))	2.16[bc]	2.13[c]	2.24[ab]	2.0[d]	2.28[a]	0.045	0.001	0.218	0.075	0.093

[1]$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, [2]$SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, [3]$SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, [4]$SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, [5]$SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.
[6]SEM: Standard error of the mean.
Contrasts: [7]$SIFC_{CO}$ vs. $SIFC_{LN}$, $SIFC_{TW,}$ SIFCSD and $SIFC_{AL}$, [8] $SIFC_{LN}$ vs. $SIFC_{TW}$, [9] $SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{TW.}$
[10]1000 - CP (g/kg) - Ether extract(g/kg) - Ash (g/kg), [11] Total carbohydrates(g/kg) - NDF ash correction (aNDF) (g/kg).
[12]NFC (g/kg)- starch (CB1) (g/kg), [13] (aNDF) (g/kg)- CC (g/kg), [14](aNDF) (g/kg) ×undigested aNDF after a 240 h in vitro fermentation and ash correction.
[a,b,c] Within rows, means with common superscript (s) are not different ($P>0.05$).

Fourier-transform infrared spectroscopy (FTIR)

Figures 1 and 2 show (single or general, respectively) FTIR spectrum in the region of 4000-400 cm^{-1}. Wavenumber peak of amide I (1700-1600 cm^{-1}) and amide II (1,577 to 1,486 cm^{-1}) were larger for $SIFC_{CO}$ (1656.49cm^{-1}) and $SIFC_{SD}$ (1515.55 cm^{-1}) respectively. The $SIFC_{TW}$ and $SIFC_{LN}$ showed similarity in molecular protein spectral patterns (Figure 3).

Scanning electron microscopy

The starch granule structure was clearly seen on the examined surfaces of processed corn grains (Figure 4). The percentage of starch granule types is illustrated in Table 4. Different surfactants impacted variably on the protein matrix disturbance compared to $SIFC_{CO}$. The expansion of starch granules in processed corn grains with surfactant ($SIFC_{LN,}$ $SIFC_{TW,}$ $SIFC_{SD}$ and $SIFC_{AL}$) was higher than $SIFC_{CO}$. The order of starch granules, as well as granule deformation, changed by surfactant application. Surfactants caused wrinkling and quitting in the starch granules and occasioned the denaturation of the protein matrix. The biggest change in the morphology of starch granules was originated by the $SIFC_{TW}$ treatment. Treatments containing surfactants showed an increase in B starch granules and reduction in A and C granules.

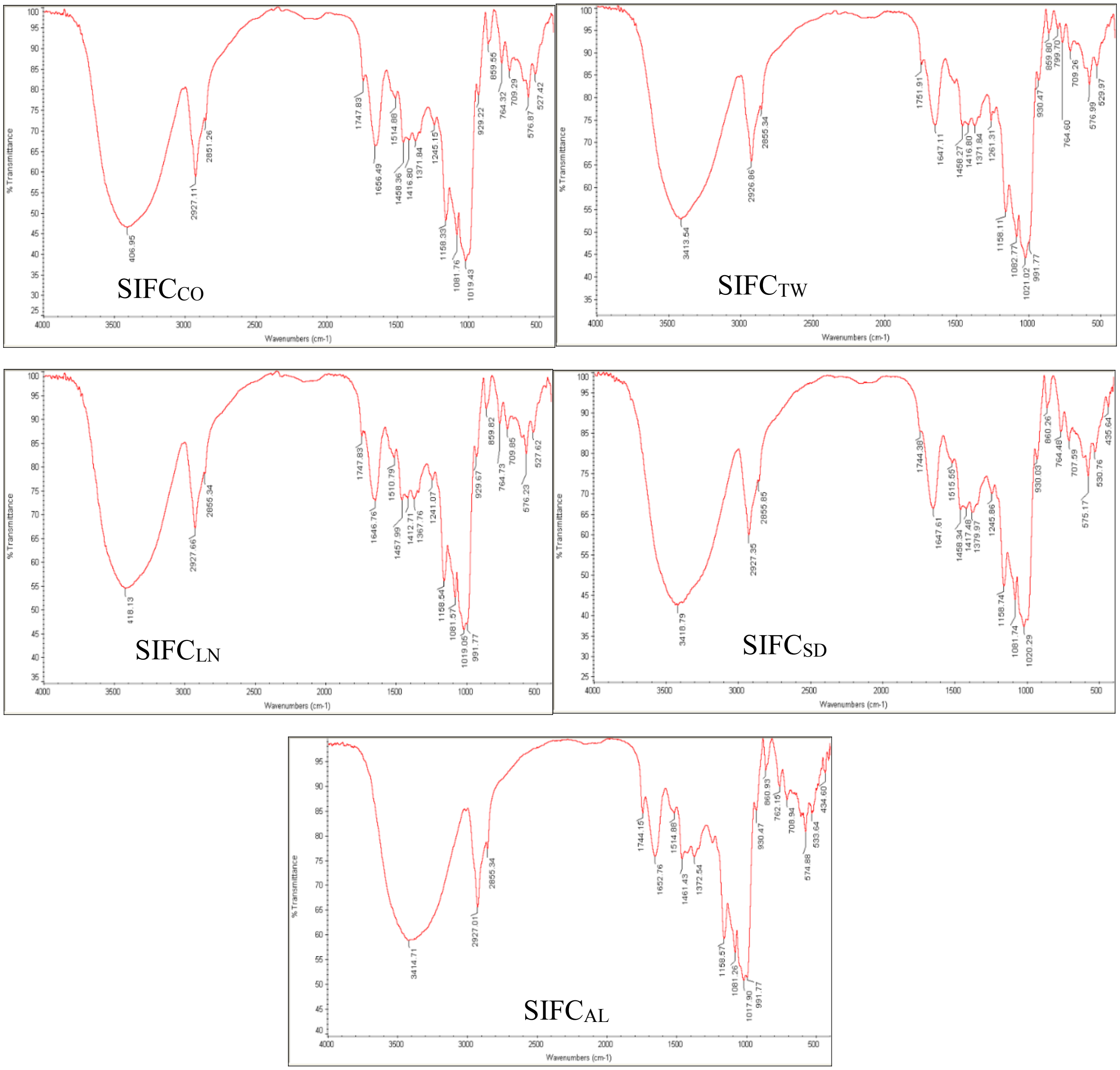

Figure 1. Typical Fourier transform infrared spectroscopy (FTIR) of full molecular spectrum of steam-infrared heated flakes of corn grains applied nonionic and ionic-based surfactants.
$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, $SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, $SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, $SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, $SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

X-ray diffraction (XRD)

The X-ray diffraction patterns are shown in Figure 5. Surfactants reduced the relative crystallinity in corn grains (Table 4). The XRD patterns indicated strong or weak and singlet or doublet peaks (2θ) in the range of 15.00◦ to 25.00◦. The highest and lowest percentages of crystallinity were pertained to $SIFC_{CO}$ and $SIFC_{TW}$ (18.97, and 16.23%, respectively).

Ruminal, post-ruminal and total tract nutrient disappearances

Ruminal, post-ruminal and total tract disappearances of DM, CP, and starch are reported in Table 5. Rumen CP and starch disappearances were significantly different between the treatments. The rumen disappearance of CP was the highest for $SIFC_{LN}$ and the lowest for $SIFC_{SD}$. Ruminal digestibility of starch was the highest for the $SIFC_{SD}$, intermediate for the $SIFC_{TW}$, $SIFC_{LN}$, and $SIFC_{AL}$, and the lowest for the $SIFC_{CO}$. The result of contrasts showed that processing with leaves of LN increased the ruminal degradability of CP compared with

TW (0.286 vs. 0.245), while processing with SD and AL decreased ruminal CP disappearance compared with TW (0.201 vs. 0.245). Applying different surfactants in processing of corn grains enhanced the ruminal disappearance of starch. Post-ruminal DM disappearance in $SIFC_{SD}$ was higher than those of other treatments. The $SIFC_{AL}$ (0.700) and $SIFC_{CO}$ (0.698) had lower post-ruminal DM disappearance compared with other treatments ($P<0.05$). The $SIFC_{SD}$ had the highest post-ruminal digestibility of CP amongst treatments (0.829 and 0.936, respectively), and the lowest one in $SIFC_{AL}$ (0.690) and $SIFC_{LN}$ (0.916). Application of SD or AL increased the post-ruminal digestibility of CP and starch compared with TW (0.759 vs. 0.732 and 0.927 vs. 0.919, respectively). Surfactant application decreased the post-ruminal digestibility of starch ($P<0.05$). Processing of corn grains with TW resulted in higher post-ruminal starch digestibility compared to processing with the leaves of LN. The highest total tract DM (0.873), CP (0.861) and starch (0.977) digestibilities were noticed in $SIFC_{SD}$. The lowest values for total tract DM and CP digestibilities were seen in $SIFC_{AL}$ (0.847 and 0.754, respectively). In addition, the total tract starch digestibility was lower in $SIFC_{LN}$ (0.969) than other treatments. Significant contrasts were observed between $SIFC_{LN}$, $SIFC_{TW}$, $SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{CO}$ so that processing of corn grains with different surfactants reduced the total tract DM disappearance. Processing with the leaves of LN increased (3.76%) the total tract CP digestibility compared to TW.

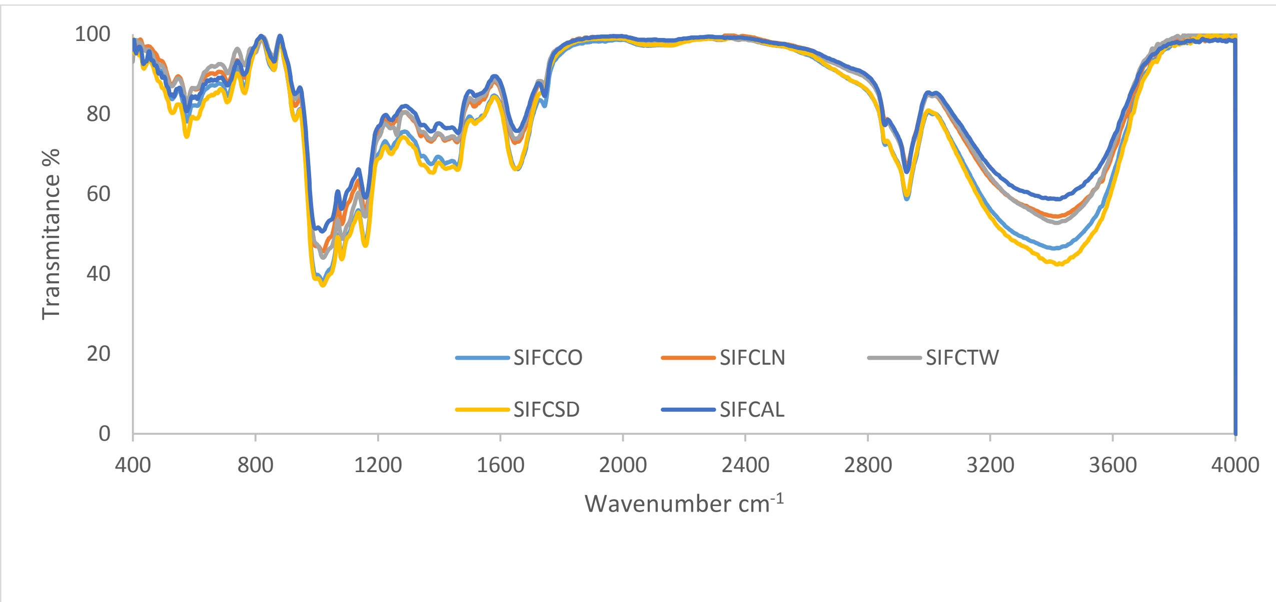

Figure 2. FTIR Spectrum of steam-infrared heated flakes of corn grains applied nonionic and ionic-based surfactants. $SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, $SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, $SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, $SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, $SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

Table 4. The effect of nonionic and ionic-based surfactants on starch granule types of steam-infrared heated flakes of corn grains

Percentage of type of starch granule	Treatments*				
		Nonionic surfactants		Ionic surfactants	
	$SIFC_{CO}$[1]	$SIFC_{LN}$[2]	$SIFC_{TW}$[3]	$SIFC_{SD}$[4]	$SIFC_{AL}$[5]
A-granules (> 15 µm)	9.7	8.5	8.4	8.9	9.1
B-granules (5-15 µm)	89.6	90.9	91.1	90.6	90.3
C-granules (< 5 µm)	0.7	0.6	0.5	0.5	0.6
Relative crystallinity (%)	18.97	17.91	16.23	18.52	18.64

[1]$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, [2]$SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, [3]$SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, [4]$SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, [5]$SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

Discussion

The present findings demonstrated that the changes in CP and WSC contents of corn grains were related to the kind of surfactant and processing. An increase in CP concentration of $SIFC_{CO}$ might be ascribed to the rupture of protein matrix and its effect on fiber fractions (lower CC proportion in $SIFC_{CO}$) during infrared heating and flaking. Rahimi et al. (2020) reported that processing of corn grains by using super conditioning-pelleting increased the CP content compared with steam-flaked corn. Infrared- radiation grain processing, increases the pressure within the seeds, because of rapid internal heating, which results in disruption of the protein matrix (Shirmohammadi et al., 2021). Processing of flaked corns with the leaves of LN caused an increase in the CP concentration compared with other surfactants (TW, SD and AL; Table 1) this was probably due to the presence of nitrogen in LN leaves. In a study conducted by Barroso et al. (2018), nitrogen content of LN leaves ranged between 0.084 and 0.14%. The $SIFC_{SD}$ had the highest WSC content among the treatments, indicating the detergent action of SD, which may be used as a surfactant.

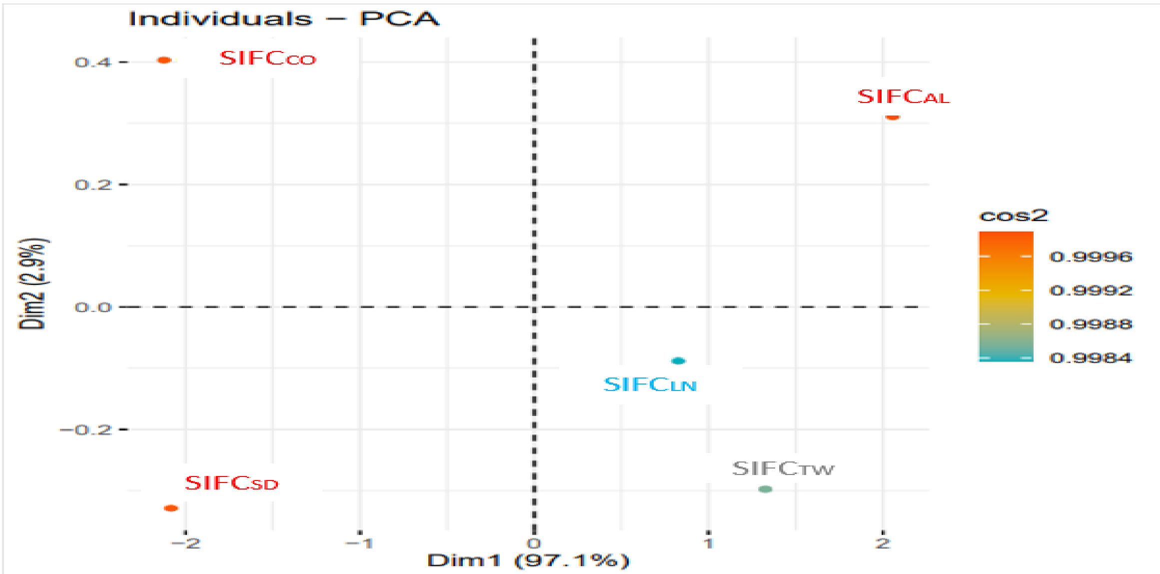

Figure 3. Principle component analysis (PCA) whole spectra of protein molecular structures of steam-infrared heated flakes of corn grains applied nonionic and ionic-based surfactants.
$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, $SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, $SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, $SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, $SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

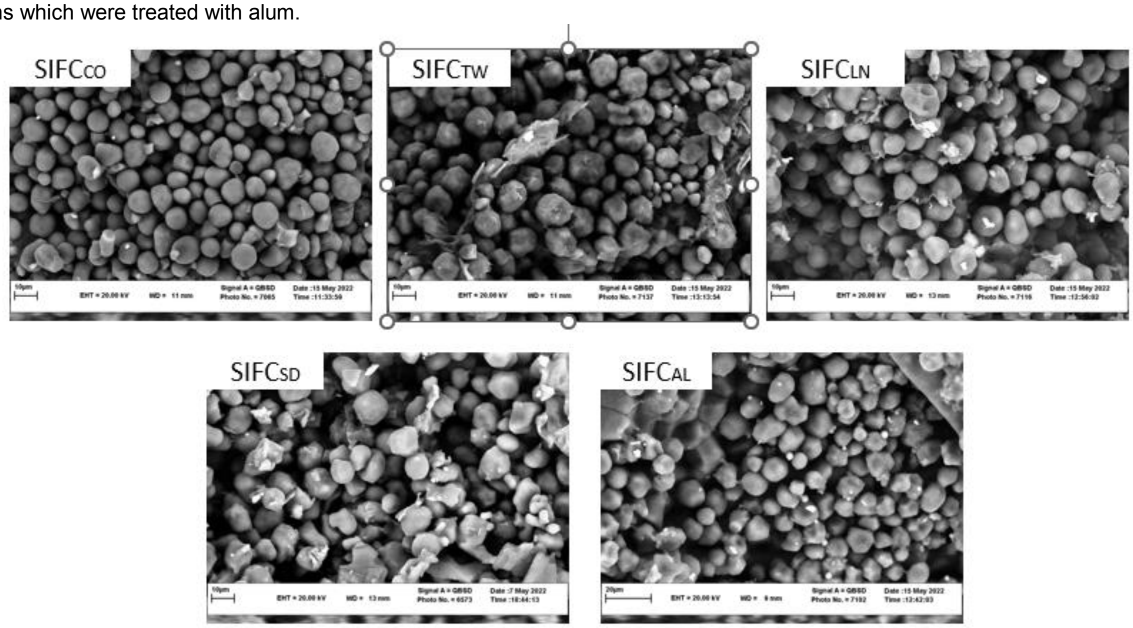

Figure 4. Scanning electron microscopy morphology of starch granules of steam-infrared heated flakes of corn grains applied nonionic and ionic-based surfactants.
$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, $SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, $SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, $SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, $SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

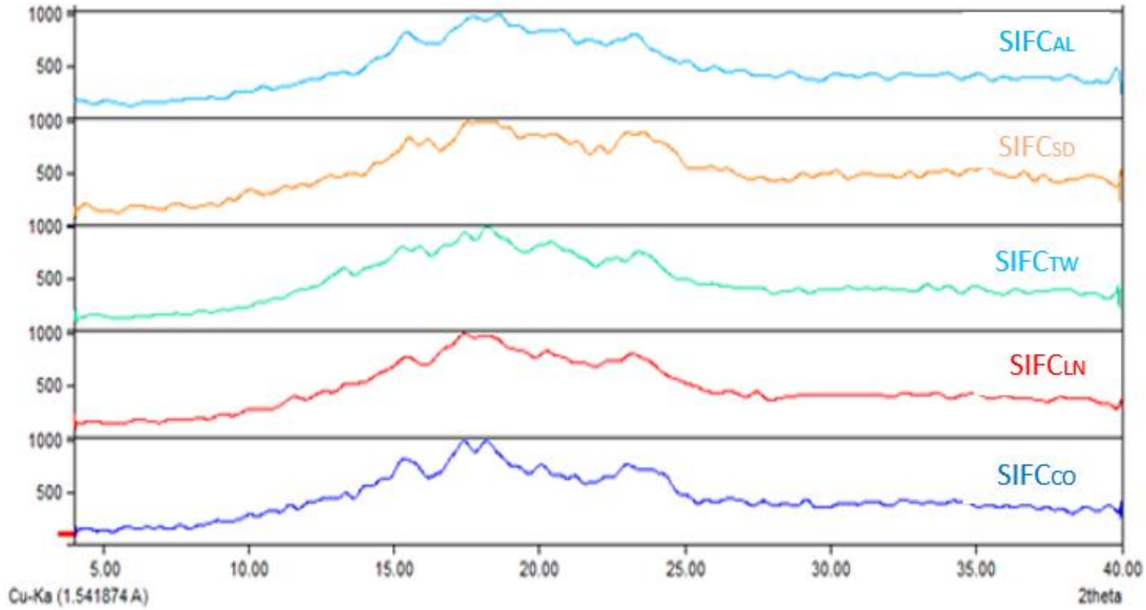

Figure 5. The X-ray diffraction pattern of steam-infrared heated flakes of corn grains applied nonionic and ionic-based surfactants.
$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, $SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, $SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, $SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, $SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.

Treatment of corn grains with various organic and non-organic surfactants; during physical processing; altered the CP and carbohydrate fractions. The $SIFC_{CO}$ had minimum fraction C, which indicated that the protein content of $SIFC_{CO}$ was not damaged by the processing. Golshan et al. (2019) reported that microwave irradiation reduced the indigestible protein fraction. Carbohydrate fractions indicated different potential for the applied organic and non-organic surfactants. Processing the corn grains with surfactants improved the B2 and B3 fractions; this might be a consequence of the surface tension, which increased water absorption, causing water to interact with the carbohydrate content, therefore, making it more accessible to digestion (Hristov et al., 2007). The SD is a surface-active compound that can affect CC fractionation. Surfactants may affect the dissolution of water-insoluble materials (Qiu et al., 2020).

According to Figures 1 and 2, treatment with surfactants caused a change in the FTIR spectrum. The primary and secondary protein structures are positively associated with CP chemical content, fractionation of CP, and quality of the *in situ* mobile bag ruminal degradation (Xin et al., 2020). The $SIFC_{CO}$ had the largest wave-number peaks of protein amide I and amide II, reflecting the greater protein concentration in $SIFC_{CO}$. The changes in the molecular structure of amides I and II in feed ingredients might have altered the quantitative CP concentration (Sun et al., 2018). The protein molecular secondary structure significantly impacts on protein quality and digestibility, with the proportion of β-sheets usually exhibiting a negative correlation with protein digestibility (Yu et al., 2004, Peng et al., 2014). The spectral data in this experiment indicated that the $SIFC_{SD}$ showed an individual wave-number peak of β-sheet at 1638 cm^{-1}, different from others. The PCA results indicated that the protein molecular spectra of $SIFC_{CO}$ were distinct from the spectra of flaked corn grains processed with surfactants. Thus, it can be inferred that heat processing, accompanied by surfactant, alters the CP solubility and degradability.

The type of grain and the intracellular components have an effect on the size of the starch granules and gelatinization; so that any increase in the degree of starch gelatinization results in an increase in starch digestibility (Ren et al., 2022). Besides, any change in the shape of starch granules, because of chemical treatments, such as swell and crinkle, may present a reflex regarding starch gelatinization. In this experiment, the surfactants intensified the disruption of starch granules (Figure 4). Surfactants enhance water penetration into processed corn grains, escalate the shapelessness of starch granules, and also provide more water for gelatinization. The availability of corn starch increased when organic and non-organic surfactant were added to the grain during physical processing (Sindt et al., 2006).

The crystalline structure of the starch granules was reduced by surfactants. Processing under microwave irradiation decreased the corn starch crystallinity 30.1% to 23.2% (Wang et al., 2019). The starch crystalline form can be divided into A, B, C, or V types (Van Hung et al., 2016). The A type crystallinity, usually detected in cereal grains, peaks at 15˚, 17˚, 18˚and 23˚ 2θ (Biliaderis and Galloway, 1989). According to Dome et al. (2020), corn starch has diffraction peaks (2θ) at 15.00˚, 22.93°, and a doublet at 17.02˚ and 17.92˚. According to Oliveira et al. (2017), the extruded corn flour had a crystallinity range of 16.97-23.81%, which is consistent with our findings. The degree of retrogradation value of starch was considerably positively associated with the resistant starch content and relative crystallinity of X-ray pattern (Wang et al., 2019). Combination of corn grains and surfactant reduced the water surface tension, improved water absorption, and increased the moisture content; as a result, starch gelatinization increased and granule crystallinity decreased.

Table 5. The effect of nonionic and ionic-based surfactants of steam-infrared heated flakes of corn grains on ruminal, post ruminal and total tract disappearances of dry matter, crude protein and starch using *in situ* mobile nylon bag technique

Parameters	Treatment					SEM[6]	P-value			
	$SIFC_{CO}$[1]	Nonionic surfactants		Ionic surfactants			Treatment	Contrasts		
		$SIFC_{LN}$[2]	$SIFC_{TW}$[3]	$SIFC_{SD}$[4]	$SIFC_{AL}$[5]			C[7]	C[8]	C[9]
Dry matter										
Ruminal	0.472	0.486	0.484	0.492	0.494	0.017	0.775	0.0528	0.180	0.728
Post- ruminal	0.698^{b}	0.731^{ab}	0.719^{ab}	0.75^{a}	0.70^{b}	0.019	0.0055	0.0226	0.184	0.083
Total tract	0.839^{b}	0.862^{ab}	0.854^{ab}	0.873^{a}	0.847^{b}	0.012	0.0059	0.007	0.191	0.093
Crude protein										
Ruminal	0.250^{ab}	0.286^{a}	0.245^{ab}	0.187^{c}	0.215^{cb}	0.025	0.0011	0.101	0.044	0.029
Post- ruminal	0.705^{cd}	0.758^{b}	0.732^{bc}	0.829^{a}	0.690^{d}	0.017	<0.001	0.515	0.108	0.057
Total tract	0.777^{cd}	0.827^{b}	0.797^{bc}	0.861^{a}	0.754^{d}	0.016	<0.001	0.273	0.050	0.041
Starch										
Ruminal	0.621^{b}	0.639^{ab}	0.638^{ab}	0.649^{a}	0.642^{ab}	0.0122	0.0463	0.044	0.502	0.109
Post- ruminal	0.927^{b}	0.916^{e}	0.919^{c}	0.936^{a}	0.918^{d}	0.000	<0.001	0.006	0.002	<0.001
Total tract	0.972^{b}	0.969^{d}	0.970^{c}	0.977^{a}	0.970^{c}	0.001	<0.001	0.273	0.249	0.068

[1]$SIFC_{CO}$) Steam-infrared heated-flakes of corn grains, [2]$SIFC_{LN}$) Steam-infrared heated-flakes of corn grains which were treated with leaves of *Laurus nobilis*, [3]$SIFC_{TW}$) Steam-infrared heated-flakes of corn grains which were treated with Tween80, [4]$SIFC_{SD}$) Steam-infrared heated-flakes of corn grains which were treated with sodium dodecyl sulfate, [5]$SIFC_{AL}$) Steam-infrared heated-flakes of corn grains which were treated with alum.
[6]SEM: Standard error of the mean.
Contrasts: [7] $SIFC_{CO}$ vs. $SIFC_{LN}$, $SIFC_{TW}$, SIFCSD and $SIFC_{AL}$, [8] $SIFC_{LN}$ vs. $SIFC_{TW}$, [9]$SIFC_{SD}$ and $SIFC_{AL}$ vs. $SIFC_{TW}$.
[a,b,c] Within rows, means with common superscript (s) are not different (P>0.05).

Surfactants application of corn grains altered the ruminal nutrient disappearance. Assessment of the disappearance characteristics of feedstuff by *in situ* mobile nylon bag technique may provide valuable information (Allen, 2015). Barley grains which were treated with AL showed an increased in both post-ruminal and total-tract starch disappearance (Naseroleslami et al., 2018). They explained that the grain processing with AL may affect the viscosity and fiber fraction; therefore, regulate ruminal degradability of starch. Our results showed when corn grain was physically processed and AL applied during steam cooking, post-ruminal digestibility of starch declined. The differences in starch digestibility obtained in our experiment compared with those reported Naseroleslami et al. (2018) might be attributed to two factors; (1) the type of grain used, and (2) the implementation of complementary physical processing in addition to the use of AL. Using ionic surfactants like SD, which have low toxicity, shelf durability, and low production costs, can be a reliable alternative for chemical processing of cereal grains (Ma et al., 2016; Simoni et al., 2020). In addition, alteration in ruminal microorganism population may change the substrate accessibility and increase the rate and extent of rumen degradation. Simoni et al. (2020) reported that *in vitro* DM degradability of corn grain which was treated with TW improved due to increased microbial activity in the rumen. Therefore, TW has a potential for modulating the rumen microbial community (Liu et al., 2013). We observed the highest ruminal CP digestibility in $SIFC_{LN}$. It may be attributed to its nonionic effect on increasing water penetration resulting in higher protein solubility. Post-ruminal, as well total tract, nutrient digestibility (DM, CP and starch) was improved when SD was used during steam cooking. Abdul-Rahman et al. (2010) found that applying SD improved microbial mass performance and ATP production in the rumen, while reduced the concentration of total short-chain volatile fatty acids and ammonia nitrogen (N-NH_3). This may explain why $SIFC_{SD}$ demonstrated higher nutrient digestibility compared with other treatments. Another reason for higher CP and starch digestibility in $SIFC_{SD}$ may be explained by the impact of SD on surface availability of substrates. Besides, it is a cation exchanger which may alter electrical conductivity (Soltan et al., 2021); these properties affect the rate of attachment of microorganisms to the fiber and increase the amount of digested substrate (Mi et al., 1983). These factors collectively indicate a reliable potential for SD to be regarded as an ionic surfactant (Costa et al., 2019). Addition of SD to rice starch has been demonstrated to decrease the extent and rate of retrogression and gelatinization temperature (Qiu et al., 2020). By reducing the surface tension and interacting with amylopectin molecules, SD can stop starch retrogression and increase starch digestibility (Gujral et al., 2003).

Conclusions

The present results demonstrated the valuable effect of surfactants, either as nonionic or ionic-based, on steamed-infrared heated-flaked corn grains, in view of the nutrient composition as well as digestion, and molecular morphology. Physical processing of corn grains combined with the application of surfactants altered the CP and carbohydrate fractions, in benefit of an increase in highly digestible fractions. This can be attributed to various changes in FTIR protein molecules and increased surface area of starch granules as observed in scanning electron micrographs. In addition, different functions of both nonionic-based and ionic-based surfactants used in the present study provide new topics that need to be considered in future experiments.

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Conflict of interest

The authors confirm that the study was conducted in the absence of any commercial or financial relevant that could be interpreted as a potential conflict of interest.

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