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Improvement of Functional Properties of Glycated Buffalo Casein Conjugated with Different Sugars Through Maillard Reaction

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Abstract: Casein, the major milk protein component and its caseinate derivatives have physicochemical, functional and nutritive properties which make them useful worldwide. So, the aim of this work was to use GRAS (generally recognized as safe) food materials in the reductive glycosylation of proteins to modify the functional properties of buffalo milk casein. The Maillard reaction involves the condensation reaction between amino acids or proteins with reducing sugars. The Maillard reaction of Rib- Glu- Gal- and Lac-casein was generated at 60°C, pH 6.5 for 72 h and dialysis for 36 h. Results revealed that lysine, arginine and histidine side chain in control casein were high and decreased for glycated casein with ribose, glucose, galactose and lactose, respectively. Casein glycated with galactose showed the highest buffer index at pH range 7.0-8.0, followed by glucose, then ribose. For the foam stability, results indicated gradual decrease up to 60 min of experimental time. With all pH values used (2 to 10), increasing the time of setting, the foam volume stability generally decreased. The glycated casein with lactose showed the best foam stability at pH 6.0. The emulsion stability decreased by increasing the time of storage. A significant difference between type of carbohydrate and emulsion properties was observed whereas, glucoglycation possessed the highest significantly value of casein emulsion stability.

Key words: Ribose · Glucose · Galactose · Lactose · Glycosylation

INTRODUCTION

As the world population grows, there will be an increased demand for food materials with new functionality and, if necessary, desirable functional properties which can be incorporated in proteins through modifications.

Such modification might make non-conventional food proteins more applicable for human consumption. Protein modification usually refers to internal changes in protein structure by physical, enzymatical or chemical agents [1]. The hydrophilic / hydrophobic balance or net charge at the protein surface may be changed by glycosylation. Changes in these properties may result in a modified isoelectric point and / or conformation, which in turn will influence the overall functional behaviour of the protein. Specific functional characteristics that can be affected by chemical modification include solubility, surficial properties, degree of hydration, tendency for gelation and thermal stability [2]. A number of chemical methods for the attachment of carbohydrates to proteins have been reported. These include coupling through Maillard based reaction [3], reductive alkylation [4] and carbodimide-promoted amide formation [5]. Direct reductive coupling of carbohydrates to the ε-amino groups of lysyl residues has been widely used. Up to now, the use of chemically modified proteins as food additive was hampered by the toxic character of most of the necessary chemical reagents [6]. The Maillard reaction was used to improve the functional properties (solubility, heat stability, emulsifying and foaming properties) of β-lactoglobulin glycated with several sugars (arabinose, galactose, glucose, lactose, rhamnose or ribose) [7]. So, the aim of this work was to use GRAS (generally recognized as safe) food materials in the reductive glycosylation of proteins to modify the functional properties of buffalo milk casein. Whole Egyptian buffalo casein, which contains from 9 to 24 lysyl

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groups per molecule [8], has been selected as a model protein for this study in N-alkylation of proteins. Glycosylation of casein was performed with glucose, galactose, ribose or lactose. Such modification is essential for increase the function properties of casein in order to increase the economical return of milk processing.

MATERIALS AND METHODS

Buffalo milk samples were obtained from the herd belonging to the Experimental Farm, Animal Production Department, Faculty of Agriculture, Cairo University. Fresh milk was skimmed twice using milk separator. Casein was separated from buffalo skim milk by 1N HCl at pH 4.6 and glycosylated using different sugars (Glucose, Galactose, Lactose or Ribose) at 60°C for 72h at pH 6.5. The modified and unmodified proteins were stored under freezing. All chemicals used in the investigation such as D-glucose, D-galactose, D-lactose and D-ribose monohydrates were obtained from Sigma Chemical Co. All other reagents were of analytical grade.

Glycosylation of Casein: The method of Chevalier *et al*., [7] was adopted for the glycation of casein. Casein (1.5 g protein / L) and the different sugars (0.217 mol / L) were dissolved in 0.1 mol / L phosphate buffer, pH 6.5. After filtration, mixtures of protein and sugars were put in tightly- capped flasks and heated in a water bath at 60°C for 72h. All experiments were performed under strictly anaerobic and sterile conditions; all media were purged and saturated with N_2. After heating, the different fractions were dialysed for 36 h at 4°C against distilled water to remove unreacted sugars. The glycosylated and unglycosylated caseins were stored at -20°C. Properties of the modified and unmodified protein were studied after dissolving it at 0.1% protein content using 1N NaOH solution.

Total Protein: Total protein in the casein (acid or glycosylated) was determined by Micro-Kjeldahl method as mentioned by AOAC [9] using nitrogen conversion factor of 6.38.

Determination of Amino Acids Composition: Amino acids other than tryptophan for modified and unmodified casein were determined with a "High performance amino acid Analyzer" as described by Moore *et al*. [10] and Kirsten and Eggum [11]. LC 3000 amino acid analyzer was used in which a sample of 50 µL was injected, using column Na-E/F/D 25 cm. The complete analysis took about 78 min. The level of amino acids was calculated depending on the peaks obtained from the amino gram sheet.

Buffering Capacity and pH Value: Buffering capacity and initial pH values were determined by the method of Morr *et al*. [12], as follows: Sufficient casein was dispersed in 100 ml distilled water to provide a 0.1% protein concentration. The initial pH value was determined with a HANNA pH meter. The dispersions were then titrated to pH between 3 – 10 with 0.1N HCl or o.1N NaOH. Buffer capacity was calculated for each 1.0 pH change Δ pH by the expression:

$$dB/dpH = \text{meq titrant} / \text{wt of protein (g)} \times \Delta \text{ pH}$$

Functional Properties of the Glycosylated Casein

Foam Volume Capacity and Foam Stability: Foam volume capacity (FVC) and Foam stability (FS) were adopted according to the method of Patel *et al*. [13]. Accurately, 100 ml of 0.1% of the casein samples were stirred and the pH was adjusted to the desired level using either HCl or NaOH 0.1N. After that the protein solution was whipped for 5 min using electric blender (Moulinex, Superman 150) at the speed setting "3". The total volume was recorded and the foam volume capacity (FVC) was expressed as percentage of the developed foam volume in relation to the initial volume of used liquid sample according to the following equation:

$$FVC\% = \frac{\text{Initial volume of foam including liquid (ml)-volume of sample used(ml)}}{\text{Volume of liquid sample used (ml)}} \times 100$$

The foam stability (FS) was indicated by following up the changes occurred on FVC after time intervals of 5min up to 25 min.

Emulsifying Capacity and Emulsion Stability: Emulsifying capacity and stability were done according to the method of Pearce and Kinsella [14]. A measured amount of pure corn oil (25 ml) and 75 ml of aqueous protein solution (0.1% protein) were shaken together in a blender for 3 min at room temperature. One milliliter of the emulsion was diluted in 100 ml volumetric flask by 0.1% sodium dodecyl sulfate (SDS) solution. The absorbency of the diluted emulsion was then determined in a 1 cm path length cuvette at 500 nm using spectrophotometer, (Spicoll II). Emulsifying capacity (EC) was determined as emulsifying activity index (EAI – m^2g^{-1}).

$$EAI\ (m^2\ g^{-1}) = \frac{2 \times 2.303 \times \text{absorbence at 500 nm}}{25 \times \underset{\text{(in meter)}}{\text{pathlength of cuvette}} \times \underset{\text{(g/m3)}}{\text{Protein conc.}}}$$

The emulsion under test was held at room temperature and periodically after 1, 2, 3, 4 and 5 days, aliquates of the emulsion were taken for dilution and absorbency was measured as described above to calculate emulsion stability.

Statistical Analysis: Statistical analyses were performed using a SAS program [15]. Duncan's multiple range test was used to determine significant difference ($P<0.05$) among treatments after initial demonstration of a treatment related effect by analysis of variance [16].

RESULTS AND DISCUSSION

Amino Acid of Native Buffalo Casein and Glycated Casein with Different Sugars: Certain protein groups are particulary prone to glycation, they include terminal amino groups and lysine side chains [17]. Arginine side chains can be glycated as well [18]. Table 1 shows the amino acids (mg/100 mg) of control and glycated caseins with different sugars used in our study. From these results, it can be seen that the most affected amino acids group side chains was lysine, arginine and histidine. Lysine side chain in control casein was 8.5 mg and decreased to 2.0, 3.4, 4.6 and 3.49 mg for glycated casein with ribose, glucose, galactose and lactose, respectivity. These results indicated that lysine glycation may be occurred within the ratio of 45 to 75%. Histidine side chain in control casein was 2.98 mg and decreased to 0.001, 1.49, 1.15 and 1.7 mg for glycated casein with ribose, glucose, galactose and lactose, respectivity. These results indicated that histidine glycation may be occurred within the ratio of about 40 to 100%.

Ashoor and Zent [19] classified all amino acids comprising natural protein and reducing sugars during thermal processing of foods into three groups according to the Maillard browning produced when they are heated with common reducing sugars. The first group includes lysine amino acid. Arginine ranks in the third group which includes the low browning producing L- amino acids.

Buffer Capacity of Glycated Buffalo Casein with Different Sugars: It is well known that buffer solution is able to retain almost constant pH when small amount of acid / base is added. Quantitave measure of this resistance to pH change is called buffer capacity. The buffer capacity definition that takes this intuition into account is given by dB/dpH which was determined mathematically using buffering intensity formula given by Van Slyke [20]. The statistical analysis using SAS program was used for evaluating the data and its results are given in Fig. 1. It can be seen that casein glycated with galactose showed the highest buffer index (0.035), followed with glucose (0.029), then ribose (0.025) and the lowest was with lactose (0.016). The buffer capacity was highest at pH 7.0 -8.0 > 6.0 -7.0 > 4.0-5.0 > 3.0 - 4.0 > 5.0 – 6.0 > 8.0 – 9.0 > 9.0 – 10.0. It seems that the changes in casein composition and structure as a result of glycation could cause significant changes in the buffer capacity of casein solution at the same pH range (pH 7.0 – 8.0).

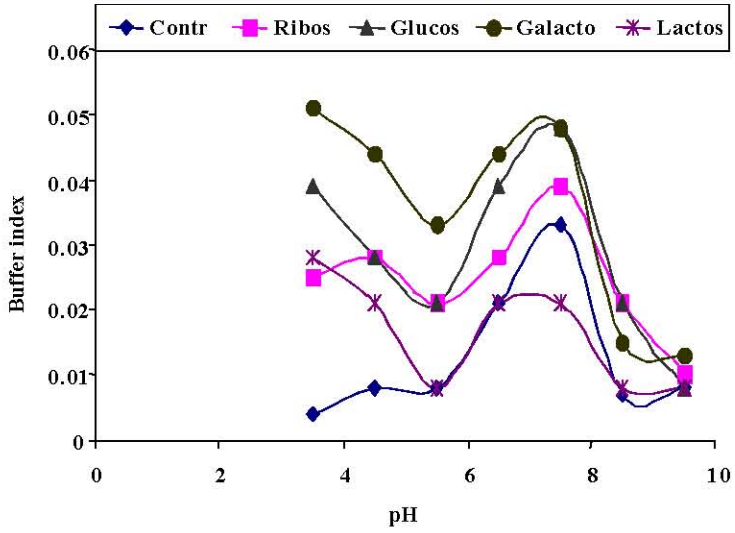

Fig. 1: Buffer capacity of unglycated and glycated buffalo casein with different sugars

Table 1: Lysine, arginine and histidine side chains of glycated buffalo milk casein

Casein treatments	Lys mg/100mg protein	% of glycation	Arginine mg/100mg protein	% of glycation	Histidine mg/100mg protein	% of glycation
Control casein	8.522	0.00	3.530	0.00	2.983	0.00
Riboglycated	1.997	76.57	1.515	57.08	0.001	99.97
Glucoglycated	3.412	59.96	1.407	60.14	1.494	49.92
Galactoglycated	4.633	45.63	1.284	63.63	1.155	61.28
Lactoglycated	3.494	59.00	1.493	57.71	1.701	42.98

Table 2: The effect of modification of glycated buffalo casein on its foaming volume capacity (%) at different pH values

pH	Glycated casein treatments					
	Control casein	Ribose	Glucose	Galactose	Lactose	Total mean of each pH for all treatments
2	32.50	67.97	78.6	56.70	75.50	59.01 BC
4	41.27	54.97	130.8	53.17	81.47	69.07 AB
6	68.77	42.27	133.3	82.90	90.27	81.67 A
8	61.27	12.6	150.0	91.17	80.87	74.11 AB
10	56.27	6.77	150.0	43.70	14.6	46.49 C
Total mean of each sugar for all pH values	52.01 D	36.92 C	107.35 A	65.53 B	68.54 B	

Means with the same letter are not significantly different.

Foaming Properties of Glycated Buffalo Casein with Different Sugars: Foam is two phase system in which a distinct gas bubble phase is surrounded by continuous liquid lamellar phase. Proteins are the most useful surface active agents for use in food where stable foam and foam capacity are required such as in the case of whipped cream and ice cream. The tendency of the solution to form a foam, usually termed "foam volume capacity" (FVC) and once the foam has formed, its stability over time is termed "foam stability" (FS).

Foam Volume Capacity of Modified Casein with Different Sugars: The foam volume capacity values of modified and unmodified buffalo casein with different sugars are given in Table 2. The foaming volume capacity was increased by modification of casein with ribose, glucose, galactose and lactose at all pH values. Modification of casein with glucose display the highest foaming capacity out of all the modification of casein treatments, being 78.6% at pH 2 and reached to 150% at pH 10.0. Unmodified casein had the lowest foam expansion compared with modified protein, being 32.5% at pH 2 and 56.27% at pH 10.0. These results clearly indicated that modification of casein with lactose come next to glucose in this respect, but up to pH 6. It can be seen also that casein glycated with ribose lost its FVC over pH 6.0. However, the analysis of variance of the obtained data (Table 2) proofed that FVC was significantly affected by different sugars used in casein glycation and glucose possessed the highest significant effect.

The same analysis of variance showed that the FVC is significantly affected by varying the pH value and the highest FVC mean percentage is attained with pH 6 followed by pH 8. At these pH values, the rigidity of the interfacial film is very important due to maximum protein-protein interaction as reported by Cheftel *et al.* [21], so that, the foaming stability is enhanced.

Foam Stability (FS) of Modified Buffalo Casein with Different Sugars: The foam stability values at different pH values of modified and unmodified buffalo casein using ribose, glucose, galactose and lactose are given in Table 3. Control casein showed a gradual decrease in foaming stability up to 35 min at pH 2.0. For pH 4.0 the FS decreased gradually up to 40 min. At pH 6.0, it showed the highest foaming volume and still the highest among all the times studied for control buffalo casein. Modification with ribose inhibited the foaming during the period of 60 min and at pH 8.0 and 10.0 it was completely not stable. Glycated casein with glucose, galactose and lactose improved the foaming stability. However, glucosglycation appeared to be the best condition for obtaining foam with better stabilities at different pH ranging from pH 2.0 to pH 10.0. Results indicated a gradual decrease up to 60 min of experimental time. With all pH values used, increasing the time of setting, the foam volume stability percentage generally decreased up to 60 min. The foam volume after the first 5 min decreased to one third the original volume and after 10 min it decreased to one fifth only. After that, the decrease was slower and reached about 1/10 from

initial volume between 40 - 45 min. Glycated casein with glucose presented the highest values of foam volume and stability at pH 6.0 followed by casein glycated with lactose. Glycated casein with ribose and galactose presented the lowest values upto the end of the experiment.

The improvement in foaming capacity of the glycated caseins was related to the increase in solubility or to the change in molecular structure induced by glycation or to both conjugated effects. The behavior of proteins at the air/water interface has been described by many authors [7, 22-27]. For adsorption, protein must follow some successive steps. First, diffusion from the bulk solution to the interface. This stage depends essentially on the size of the hydrated molecule. Monomers of casein probably adsorb more easily than aggregates, because of smaller size and the higher availability of their residues. Second, overcoming the electrostatic barrier to adsorb, the higher charge of the molecule, the higher the energy required. Third, adsorption, which depends mainly on the proportion polar residues, on their repetition along the chain and the flexibility of the macromolecule.

The conformational rearrangements for surface coverage and loop and tail formation are easier when molecules are flexible. Later, film formation at the interface whose thickness and resistance depend on the tertiary structure of the protein – protein interaction [28].

It is well known that, a number of moleculars such as mass, conformation, net charge and hydrophobicity of proteins were shown to play important roles in the definition of their functional properties [29]. Particularly, glycation can be an efficient method to increase hydrophilicity of proteins and modifies their solubility, conformational stability [30].

Emulsifying Properties

Effect of Buffalo Casein Glycation on Emulsifying Activity at Different pH Value: Emulsifying properties of buffalo casein and modified glycated casein with ribose, glucose, galactose and lactose were studied at different pH values and the results are presented in tables 4 & 5. Emulsifying activity index (EAI) at zero time of control casein was ranged between 40.97 and 121.11 m^2g^{-1}. For riboglycated casein the range was 171.65- 248.80 $m^2 g^{-1}$, whereas the respective ranges were 179.64 - 276.18 m^2g^{-1}, 125.29-180.34 m^2g^{-1} and 186.48-267.75 for glucose, galactose and lactose modified caseins, respectively.

The Emulsion Stability of Glycated Caseins During Storage: The emulsion stability was determined after 1,2,3,4 days for control casein and glycated caseins with different sugars and the obtained results are present in Table 6. The emulsion stability decreased by increasing the time of storage. It started with 87.70 m^2g^{-1} for unmodified casein at zero time and then decreased to reach the value of 30.57 $m^2 g^{-1}$ at the 4th day of storage. Glucoglycated casein started from 240.23 $m^2 g^{-1}$ and ended with 170.57 at the 4th day. Glucoglycated casein shows the highest value of stability among other sugars used for glycation. Emulsion activity index at the 1st day of storage was slightly decreased for all model systems with different carbohydrates glycation of caseins than at zero time (Table 6). Glucose showed highest values followed by ribose, lactose and galactose as compared with control. Unsignificant data could be seen in the case of ribo-, gluco- and lactoglycated caseins (Table 6).

After 2 day of storage glucoglycated casein showed the highest EAI of 195.48 $m^2 g^{-1}$ which was significantly differs from other glycated caseins (Table 6). The highest values of glucoglycated casein can be detected at all pH values (Table 4) then followed by lactoglycated, riboglycated, galactoglycated and unmodified casein. Also, glucoglycated casein gave the highest ESI after 3 days of storage. It was of 184.85 $m^2 g^{-1}$ followed by lactoglycated, riboglycated, galactoglycated and then control casein. Table 6 shows also a significant different values for glucose modified casein and other modified caseins as well as control casein.

At the 4th day of storage (Table 6) glucoglycated casein was significantly higher in EAI as compared with other carbohydrates. At different pH values, pH 4 shows the minimum EAI and then the EAI increased with increasing the pH up to pH 10 and with decreasing the pH to pH 2.

Statistical analysis of the obtained results was done to evaluate the effect of the type of carbohydrate, the effect of pH (Table 5) and the effect of storage days (Table 6) in emulsion properties in general.

Table 6 shows a significant difference between type of carbohydrate and emulsion properties. Glucoglycation shows the highest significantly value, 198.82 $m^2 g^{-1}$ of casein. Unmodified casein gave the least value 52.5 m^2g^{-1} casein.

Regarding the effect of pH, it could be concluded that at pH 4.0 which is near the isoelectric point of caseinates, the emulsion was at the least value (Table 5), whereas at the two sides of pH, acidic or alkaline, the emulsion increased significantly and that is may be due to the high solubility of the casein above pH 4.0.

In the respect of the effect storage period, increasing the time of storage significantly decreased the emulsion properties (Table 4).

Table 3. Foam stability (FS) of control and glycated buffalo casein at different pH values

		Foaming stability (%) during the 60 min after foaming												
Protein treatment	pH	0	5	10	15	20	25	30	35	40	45	50	55	60
Control casein	2	30.5	4.2	3.0	3.0	3.0	3.0	2.45	1.9	1.9	1.9	1.9	1.9	1.3
	4	37.5	6.6	5.1	3.6	3.6	3.6	3.6	3.6	2.7	2.7	2.7	2.7	2.7
	6	50.7	24.5	7.0	5.8	5.8	4.9	4.3	4.3	4.3	3.3	3.3	3.3	3.3
	8	47.0	9.1	5.9	4.0	4.0	4.0	3.4	3.0	3.0	2.4	2.4	2.4	2.4
	10	44.5	6.7	5.8	4.9	4.3	4.3	3.6	3.6	3.6	2.7	2.7	2.7	1.7
Glycated with ribose	2	49.5	25.0	13.9	13.9	11.1	10.1	9.6	9.6	8.6	8.6	8.6	7.6	7.6
	4	44.2	15.0	6.2	5.5	2.9	2.9	2.9	2.9	2.9	2.2	2.2	2.2	1.5
	6	38.0	10.0	8.7	8.1	6.8	6.8	6.1	6.1	6.1	6.1	6.1	3.8	3.8
	8	10.3	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycated with gluose	2	53.6	17.6	12.8	12.0	8.0	6.4	6.4	6.4	4.8	4.8	4.8	4.8	2.2
	4	67.8	14.4	14.4	14.4	12.2	11.1	8.91	4.4	4.41	4.4	4.41	4.4	3.4
	6	71.4	55.7	47.1	41.4	31.4	17.2	7.2	17.2	4.3	14.3	1.4	10.0	8.6
	8	71.6	13.7	7.4	6..3	5.3	5.3	5.3	4.2	4.2	4.2	4.2	4.2	4.2
	10	74.3	15.7	4.3	4.3	4.3	4.3	4.3	2.9	2.9	2.9	2.9	2.9	2.9
Glycated with galactose	2	46.5	4.7	4.7	4.7	4.7	4.1	4.1	3.8	3.7	3.7	3.7	3.6	3.1
	4	43.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	6	52.0	9.7	5.7	4.7	3.0	2.7	2.7	2.0	2.0	1.7	1.3	1.3	1.3
	8	57.7	8.4	4.4	3.4	3.0	2.4	2.4	2.0	2.0	2.0	1.3	1.0	1.0
	10	39.9	4.0	3.4	3.4	2.9	2.9	2.3	1.2	1.2	1.2	1.2	1.2	1.2
Glycated with lactose	2	50.1	39.9	22.6	17.3	17.3	9.3	9.3	9.3	9.3	9.3	8.9	8.9	8.9
	4	50.6	24.4	8.84	8.8	5.9	5.9	5.9	5.9	5.9	5.4	5.4	5.4	4.9
	6	58.8	50.4	3.0	33.5	31.9	30.7	21.3	21.3	20.7	20.0	19.4	19.4	18.2
	8	57.3	14.0	9.5	8.5	8.5	8.5	7.5	7.5	6.6	6.6	5.6	5.6	5.6
	10	21.1	5.7	4.9	4.9	4.9	4.9	4.2	3.3	2.3	1.6	1.6	1.6	1.6

Table 4: Effect of casein glycation with different sugars on emulsifying activity index (EAI) and emulsion stability at different pH values and during storage for 4 days

		EAI(m^2g^{-1}) at				
Casein treatments	pH	0 time	1st day	2nd day	3rd day	4th day
Control casein	2	72.83A	52.46B	43.46BC	37.34 BC	28.57C
	4	40.97A	25.67B	26.25B	14.20BC	9.83C
	6	93.13A	59.24B	40.72C	33.24D	29.00D
	8	110.44A	71.97B	60.02C	44.88D	37.79D
	10	121.11A	86.13B	73.33C	52.30D	47.82D
Glycation with ribose	2	213.66A	198.95A	175.95B	160.58BC	140.81C
	4	171.65A	165.48A	152.02AB	146.39AB	126.41B
	6	198.48A	187.58AB	164.35BC	153.84C	138.80C
	8	226.05A	208.09AB	188.88AB	161.76ABC	145.95C
	10	248.80A	241.89A	210.25AB	172.92BC	150.48C
Glycation with glucose	2	261.69A	209.11B	187.01C	179.64C	170.42C
	4	179.64A	176.04A	172.27AB	165.24C	153.84C
	6	223.85A	192.93B	190.0B	177.80D	165.25D
	8	259.78A	207.06B	199.88B	196.22B	178.19B
	10	276.18A	236.83AB	221.09BC	205.35C	185.16C
Glycation with galactose	2	165.89A	154.46B	148.02BC	140.93C	137.57C
	4	125.29A	119.15B	111.36C	108.45CD	105.68D
	6	140.48A	136.95AB	132.96BC	129.89C	123.45D
	8	155.19A	140.63AB	138.79AB	134.80B	129.51B
	10	180.34A	159.19B	143.75BC	137.73C	131.97C
Glycation with lactose	2	205.43A	191.61B	175.95C	159.37D	154.76D
	4	186.48A	177.79 A	158.45B	144.63BC	130.63C
	6	243.54A	196.64B	178.14BC	155.17BC	142.79C
	8	248.73A	214.65AB	193.89BC	169.10BC	157.84C
	10	267.75A	219.95B	203.86BC	183.30BC	161.08C
Total mean		184.70A	160.93B	147.91C	134.60D	123.34E

Means with the same letter in the same row are not significantly different.

Table 5: Statistical analysis of the effect of pH values on emulsion activity index (EAI) $m^2 g^{-1}$ of glycated casein

	Casein glycation treatments					
pH	Control	Ribose	Glucose	Galactose	Lactose	Mean
2	46.93C	177.9BC	201.57B	149.37A	177.42C	150.66C
4	23.35D	152.39D	169.40D	113.98D	159.59D	122.75E
6	51.07C	168.61CD	189.97C	132.75C	183.25BC	145.13D
8	65.02B	186.15B	208.23B	139.77B	196.84AB	159.20B
10	76.14A	204.87A	224.92A	150.60A	207.19A	172.74A

Means with the same letter in the same column are not significantly different

Table 6. The effect of modification of buffalo casein with different sugars on its emulsion activity index (EAI) m^2g^{-1} during storage for 4 days

	Casein glycation treatments				
Days of storage	Control	Ribose	Glucose	Galactose	Lactose
0 Time	87.70D	211.73B	240.23A	153.44C	230.38A
1	59.09C	200.40A	202.96A	142.08B	200.13A
2	48.76D	178.29B	195.48A	134.98C	182.06B
3	36.39D	159.10B	184.85A	130.36C	162.31B
4	30.57E	140.49C	170.57A	125.64D	149.42B
Mean ±SE	52.50 E±1.80	178.0 C±1.80	198.82 A±1.80	137.30 D±1.80	184.86 B±1.80

Means with the same letter at the same row are not significantly different.

It is possible to explain the emulsifying properties of caseinates since their primary structure has been determined. Due to their amphiphilic character and the absence of any order secondary structure, the principal α_s-casein and β-casein have an unfolded structure facilitated their adsorption at the water-oil interface. This relationship has been confirmed by Lee *et al.,* [31] who pointed out that β-casein surface properties were lost by enzymatic hydrolysis of either the very hydrophobic C-terminal fragments or the very hydrophilic N-terminal fragment.

Our results more or less agree with the results reported by Bertrand – Harb *et al.* [32] who found that glycoslation of the β-lactoglobulin improved and enhance the emulsifying properties between pH 2 and 6. It was reported also that chemical glycosylation would improve the emulsifying properties of whole caseinate by diminishing the α_{s1}- casein / β-casein association rather than improving the emulsifying properties of the casein fractions [33].

The β- lactoglobulin showed better emulsifying properties after glycation with glucose -6- P [34]. It was reported that galactose had a higher initial rate of utilization of ε–amino groups of lysine residues than lactose [35]. Also it was reported that the smaller the carbon chain of the sugar, the more reactive is the sugar with the amino group of proteins [36, 37].

According to Colas *et al.* [38], glycosylation is indeed known to increase the voluminosity of the casein fraction. Then the hydrophobic binding should be therefore reduced. It seems likely that association of casein fraction in solution would rather be responsible for the depressing effect of EAI in caseins, where as the dissociation of casein fractions due to glycation improve the emulsion properties as we have seen in our results.

CONCLUSION

The obtained results revealed that the functional properties of buffalo casein could be positively modified through glycosylation with food–safe additives. Such improvement of functional properties of buffalo casein could produce an equivalent functionality with a smaller amount of added protein, helping to reduce costs as well as to promote its use as high -active ingredient with a dilution value to develop customer-favored products with a competitive edge in the marketplace.

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