NFDM and dairy proteins antigenicity on heat treatment conditions.

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Abstract

A study to analyze the antigenicity of nonfat dry milk (NFDM) and six different kinds of dairy proteins over hot-fill treatment conditions was investigated. Two methodologies, reducing and non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis **(**SDS-PAGE) and Western blot (WB), were used to identify the target analyte (antigen) of W3501, an antiserum developed in rabbit using normal bovine whey proteins as the immunogen. The main purpose of this research was to analyze the protein profiles of dairy proteins, to determine the specific immunoreactivity of NFDM and Immunoglobulin G (IgG) when reducing and non-reducing samples undergone hot-fill treatment (95 °C for 2 min).

1. INTRODUCTION

Protein separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) can be used to determine the relative abundance of major proteins in a sample and to determine the distribution of proteins among fractions (Tsakalidou et al., 1994). Basically SDS-PAGE method separates denatured polypeptides according the molecular weight of them in the samples (Brunelle et al., 2014). The molecular weight (MW) of polypeptides can be estimated by comparison to protein of known molecular weight in SDS-PAGE. The purity of protein samples can be assessed, and the progress of a fractionation or purification procedure can be followed. Different staining methods can be used to detect rare proteins and to learn something about their biochemical properties. Specialized techniques such as Western blotting, two-dimensional electrophoresis, and peptide mapping can be used to detect extremely scarce gene products, to find similarities among them, and to detect and separate isoenzymes of proteins (De Souza et al., 2000; Hossenlopp et al., 1986).

In this study we carried out a protein profile determination, by SDS-PAGE and WB using how main sample to be studied NFDM, followed by six different whey proteins, the main purpose of this experiment was to analyze the antigenicity on protein profiles of dairy proteins, when reducing and non-reducing samples undergone hot-fill treatment of 95 °C for 2 min.

2. MATERIALS AND METHODS

2.1 Materials

The NFDM was obtained from (Saco, Middleton, WI, USA) and WPI was obtained from (Davisco Foods International, Le Sueur, MN, USA), casein (C), the purified whey proteins, Alpha-lactalbumin (-LA), Bovine serum albumin (BSA) , and immunoglobulin G (IgG) from bovine serum were obtained from (Sigma-Aldrich, St. Louis, MO), the antibody and antigen kit W3501 were obtained from (Sigma-Aldrich, St. Louis, MO) all other chemical compounds were obtained in pure grade.

2.2 Sample preparation

During sample preparation, unless otherwise specified, all subsequent manipulations were performed at 4 °C. All samples were homogenized by sonication at 11,000 rpm for 1 min twice using a ULTRA-TURRAX T25 basic homogenizer (IKA Works); all heated samples were prepared at 98 °C/600 rpm for 5 min using a Thermomixer (Eppendorf, Hamburg, Germany) and then were adjusted in the Analog Vortex Mixer (VWR), to eliminate dissolve all the evaporation drops to the top of tube and transferred to a pipet to prepare the sample that became used to load the gel.

2.3 Non-reducing and reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (WB)

Non-reducing and reducing SDS-PAGE were performed to study the protein profiles of different dairy proteins, the samples were separated by non-reducing and reducing SDS-PAGE (4% stacking gel and 15% separating gel) using a Mini-PROTEAN Tetra Cell (Bio-Rad) according Brunelle et al., 2014.

In the preparation of the Separating and stacking gel, Acrylamide/Bis at 40% solution was used (Bio-Rad company, Catalog 161-0158), 0.5 M Tris HCL such as buffer, 10 % SDS was applied to the protein samples to coat proteins in order to impart two negative charges (from every SDS molecule) to every two amino acids of the denatured protein, 10 % APS acted such as a gas and TEMED helped in the control of the polymerization time, De-GAS process was carried out in a vacuum camera and helped to avoid the formation of bubbles, and prevent the oxidation by air.

The proteins loading mass were illustrated the follows: 20 µg/lane and 10 µg/lane of NFDM, 1 µg/lane of Casein (C), 2 µg/lane of Whey protein Isolate (W), 1 µg/lane of Alpha-lactalbumin (-LA), 1 µg/lane of Bovine serum albumin (BSA), and 0.5 µg/lane of immunoglobulin (IgG), was prepared for non-reducing and reducing samples. All the samples were mixed with an equal volume (µL/µL) of the 2 × Laemmli sample buffer.

Two types of diluted samples were prepared for non-reducing and reducing SDS-PAGE, with and without Beta-mercaptoethanol (2-ME) in (0.05%) respectively, that was used as dissociating reagent to obtain degradation of co-aggregates possibly formed by disulfide interactions. Beta-mercaptoethanol (2-ME), was added, before loading. The protein profiles of the gel were visualized using Coomassie blue staining solution. All images from non-reducing and reducing SDS-PAGE were captured by the Azure C600 Imaging System and analyzed using the AzureSpot software (version 14.2, Azure Biosystems Inc., Dublin, CA, USA).

3. RESULTS AND DISCUSSION.

Even when NFDM contains all the dairy proteins presents in the samples (Fig 1), those ones were not clearly shown in the SDS-PAGE gel, this behavior was attributed to the initial condition of the NDFM that was processed in a spray dryer. (Hayashi et al., 1970; Park et al., 2016). NFDM processing produces physicochemical changes that occur as response of the heat treatment suffered on the spray drying, which affect the rehydration of NFDM powders and the functional behavior of the dispersed powder. Reason that may explain the missing of the whey proteins in the NFDM lines. Also in the NFDM lines was possible to see a low contrast between bands, which could indicate either the presence of many proteins, the low purity of them, the proteolytic degradation of the protein, or not enough migration time to separate the proteins. (Rath et al., 2009)

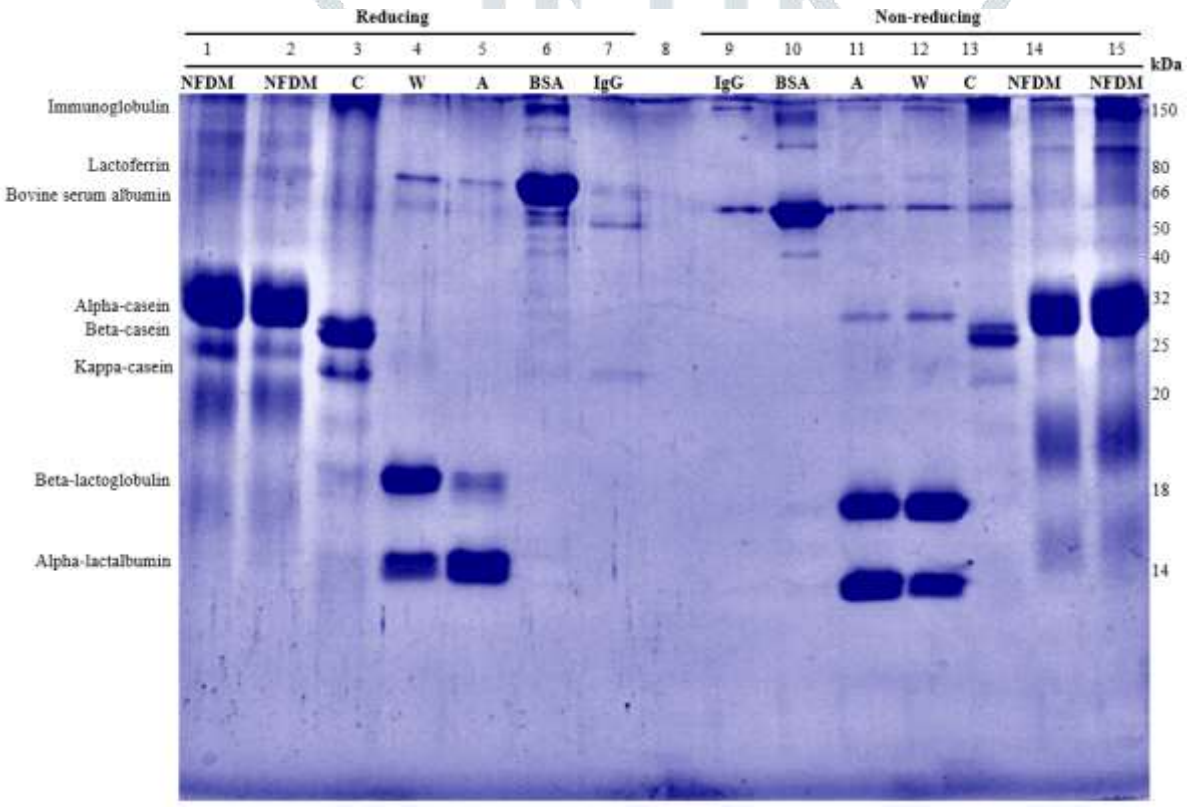

Figure 1. SDS-PAGE gel.

The denature conditions of the whey proteins, depends on the operation temperature of the spray dryer, for example: (b-LG) precipitates rapidly and selectively at high temperature (70–120 ◦C) and pH near neutral (pH-8), in the other hand (-LA) precipitates and aggregates better at acidic pH (3.5–5.5) and moderate temperature (50–65 ◦C) with long reaction times, usually accompanied by the precipitation of bovine serum albumin (BSA), immunoglobulins (Ig) and lactoferrin (Lf), while -LG and casein-macropeptide (CMP) remain soluble. (Kiesner et al., 2000; Plock et al., 1998; Fernandez et al., 2011)

Protein denaturation causes degradation bands and after further degradation produces homogeneous color or stain below the band, then produces a difficult track of the movement of the molecules in the line. The two bands of NFDM were assembled to saw the difference between the uses of a standard in high and low concentration, which is appreciable in the intensity of the line (1) and (2), the casein (C) could have the bigger contribution in NFDM, which is appreciable in the scale of reducing and non-reducing gels, also this big stain in the NFDM should be attributed to a mixture of proteins.

Reducing conditions was developed using beta-mercaptoethanol (2-ME) to reduce disulphide bridges in the whey proteins and allow that would adopt random coil conformation and better separate by size in SDS-PAGE gels. Under reducing conditions, interactions between two protein polypeptides were disrupted. However, under non-reducing conditions, the interactions are preserved. This was used approach in protein electrophoresis for their detection by Western blotting also.

The difference between reducing and non-reducing gel were represented in the intensity of the molecules, at the beginning the bands were strong but, in the wheel, track its coming to be weak, and finally disappear who shows the reducing of this compound on the line. If disulfide bond tethers together two polypeptide chains, the reducing conditions allow subunit separation. Disulfide bonds can also form within single polypeptide chain; in that case, the reducing conditions allow protein to achieve unfolded structure when denatured by heat in the presence of SDS.

According the molecular weight (MW) and tracking the movement into the gel, the biggest compound was the Immunoglobulin G (IgG), lines (7) and (9) with (150 kDa), and the little was the Alpha-Lactalbumin (-LA) lines (5) and (11) with (12 kDa), also is possible to appreciate that in the line of the immunoglobulin (igG), lines (7) and (9), that igG antibodies are large molecules of about (150 kDa) made of four peptide chains, in the line of non-reducing gel, whereas in the line of the reducing gel, and with the break of the disulfide bonds It contains was separated in heavy chains of about (50 kDa), light chains of about (25 kDa). (Shapiro et al., 1967)

The NFDM, was reduced in water in a spray dryer process, and content lactose, galactosyltransferase (GT) that it is a glycoprotein with a molecular weight varying from 35-60 kDa, depending upon the amount of glycosylation and the degree of proteolytic degradation also contain casein, that are visible in the low contrast band Image 2 (N-NR). that appear between (32 kDa and 25 kDa), the bands near to (25 kDa) that probably could be alpha-casein (-CN) with (31 kDa), beta-casein (-CN) and kappa-casein (-CN) with (19 kDa), is not proper to say that the precipitate that was obtained in the centrifuge process, (Hosseinpour et al., 2011) is casein, because the milk is a mixture that contain casein and whey proteins, if not then would be just whey (Jovanovic et al., 2007

Western blot analysis was developed to determine the antigenicity of the samples with the target antigen is appreciable that just the line (7) and (9) are present bands, this could be attributed to the FC specificity of the antibody (W3501), that was used to this study, then the line (7) that belongs to the non-reducing samples, means that the primary antibody immunoglobulin G (IgG) kept without modification in its structure with a (MW) of (150 kDa), and in the line (9) for reducing samples, the Immunoglobulin G (IgG) was separated into heavy and light chain, nevertheless, was only appreciable the heavy chain with (50 kDa) due the secondary antibody (mAb) is FC specific, and was linked just to the FC fragment. In the (Fig 2) was possible to observe one band in the line (9) that belongs to the reducing samples, the apparition of just one band that would be the heavy chain with (50 kDa), is attributed also to the FC specificity of the secondary antibody.

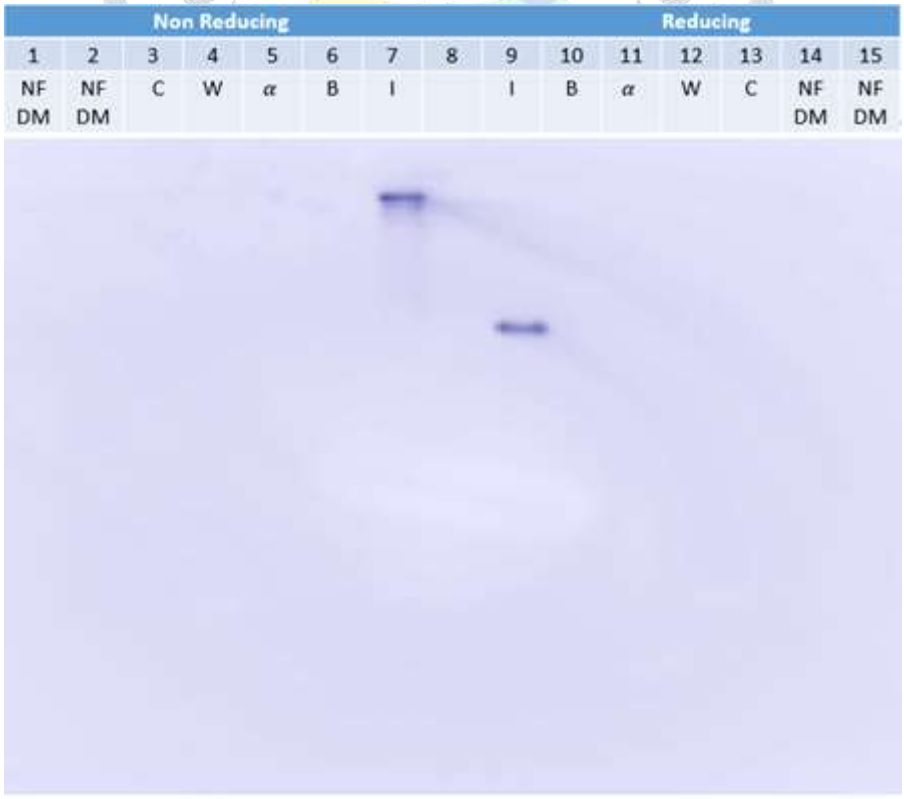

Figure 2. Western blot gel

Immunoglobulin G (IgG) was the only sample who showed immunoreactivity for Western blot, this pattern was attributed to the capacity of the (IgG) to works as an antibody. The 3D-view of the western blot analysis (Fig 3) showed the saturation of the bands, the curvature attributed to this samples means that the immunoglobulin was not overlay saturated.

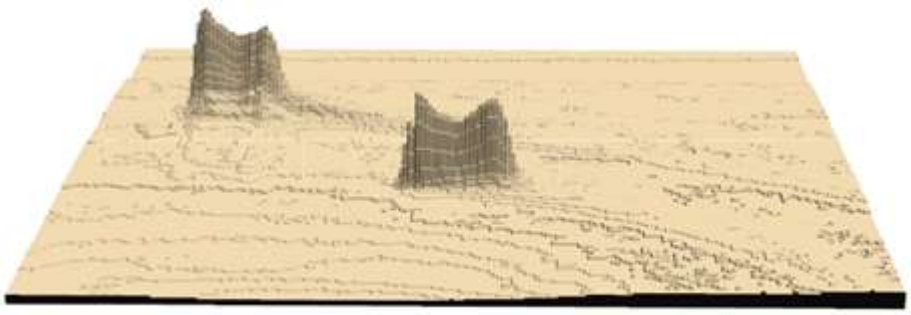

Figure 3. 3D View Western blot gel

4. CONCLUSIONS

The non-reactivity of the target analyte (antigen) with (NFDM) would be attributed to the hot-fill treatment conditions employed in this study. The antibody (mAb a2554) did not present any response in Western Blot (WB) when dairy proteins samples were analyzed. This behavior could be attributed to the FC fragment specificity of the (mAb), which showed that the antibody only bound on the antigen on a specific zone. Another fact also could be that the concentration of (IgG) in NFDM was not high enough to bind with the antibody and that probably the epitope of the IgG who should link with the antibody was destroyed in the thermal processing of the NFDM.

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