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# Structural consequences of dry heating on Beta-Lactoglobulin under controlled pH

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**Abstract**

Heating in dry state has gained a lot of interest in pharmaceutical and food industries for viral and microbial decontamination of thermo sensible products. Controlled dry heating has now become a common industrial process for improving the functional properties of food proteins. Besides this improvement, chemical modifications in protein structures involving degradation of amino acids, new intra or inter-molecular disulfide bonds, isopeptide bonds, and some other links may occur. These chemical modifications are favored by severe heating under neutral or alkaline conditions, and except disulfide interchanges, are usually not predominant during heat treatments in solution and their occurrence is not well understood. Understanding chemical modifications in protein structure that occur during dry heating of protein powders is a prerequisite for reproducible properties of final products at industrial scale. In the present work, we focused on how dry heating under acidic pH conditions affects the chemical modifications and denaturation/aggregation reactions of whey proteins. Model whey protein (β-lactoglobulin) powders obtained from freeze drying of protein concentrates adjusted to pHs 2.5 & 6.5 were adjusted to a fixed water activity (Aw 0.23). The samples were dry heated at 100°C for up to 24 hours and structural modifications induced during dry heating were followed. We showed that whatever the pH value, proteins were characterized by irreversible mass losses of 18 Da. Such mass losses were increased at lower pH value. Dry heating mainly generated small aggregates (dimers and oligomers). Strikingly, at pH 2.5 intermolecular disulfide bonds were the only crosslinks between proteins in the aggregated forms while covalent crosslinks other than disulfide bonds also participated at pH 6.5. β-Lactoglobulin hydrolysis was also detected at pH 2.5 and some of the peptides were incorporated in the oligomers. These results underline that chemical modifications in protein structures induced by dry heating are highly pH dependent. Hence, strict control of pH conditions for dry heating is indispensable to give reproducible functionality in products where such modified proteins are incorporated as ingredient.



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*Keywords*: β-Lactoglobulin; Dry-heating; pH; Protein crosslinking; Protein hydrolysis

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## 1. Introduction

Proteins are widely used as ingredients in food products due to their ability to generate large diversity of textures (gel, foam and emulsion). Modification of protein structures strongly affects this aptitude. Controlling the denaturation/aggregation level of proteins, i.e. pre-texturization, is of tremendous importance for tuning their functional properties.

Industrially, dry-heating has been proved to be a promising process to improve the functional properties of proteins by pre-texturizing them [1], which reduces the effective cost by optimizing the quantity of input. However, non reproducible functional properties are generally observed for industrially produced dry-heated proteins. In fact, thermal intensity for dry-heating (temperature and time for dry heating) is usually the only parameter that is modified to reach the expected denaturation/aggregation level of proteins. Other parameters of the powder such as Aw and pH are also able to tune the denaturation/aggregation kinetics of proteins as well as the type of modifications induced in the powder.

In a previous work, Gulzar et al. [2] characterized the effect of pH on the dry heat-induced denaturation/aggregation of commercial whey protein isolate. We found that the kinetic of denaturation/aggregation of whey proteins was increased by increasing the pH of dry heating. In addition, the pH also affected the size and solubility of aggregates as well as the interactions among the protein molecules involved to form aggregates.

In order to progress on the structural changes induced by dry-heating, purified samples of major whey protein β-Lg adjusted at pH 2.5 and 6.5 and water activity ($a_w$) 0.23 were dry heated at 100°C for up to 24 hours (dry heating conditions were same as taken for WPI analyzed previously). The structural modifications induced during the course of dry heating were investigated.

## 2. Material and Methods

### *2.1 Materials*

Spray dried β-Lg (industrial source) contained 93% protein (determined by Kjeldahl method), $4.3 \pm 0.2$% moisture and about $0.89 \pm 0.04$% mineral salts. Glycine was from Acros Organics (Geel, Belgium), all other chemicals were from Sigma Aldrich (Saint-Quentin-Fallavier, France).

### *2.2 Preparation of powders*

Spray-dried β-Lg powder was dissolved in distilled water at a protein concentration of 15% and the solution was adjusted to two different pH's (2.5 and 6.5) using HCl. The solutions were then lyophilized. The samples containing 10 g of powder were stored for two weeks in desiccator having saturated salt ($CH_3CO_2K$) solution to reach an $a_w$ of 0.23. Before dry heating, the $a_w$ of powder was checked by $a_w$ meter (Novasina RTD 200/0 and RTD 33, Pfäffikon, Switzerland).

### *2.3 Preparation of samples*

Powders ($a_w$ 0.23) with two different pH's were heated at 100°C up to 24 hours in hermetically sealed bottles. Then, all the powders were reconstituted at 10 g $L^{-1}$ in distilled water containing NaCl in order to reach the same final ionic strength (0.12 M) in all samples. The pH was adjusted to 7 by addition of 1 N NaOH. These samples were used to characterize the changes induced by the dry heat treatment.