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# The impact of alkaline conditions on storage proteins of cereals and pseudo-cereals

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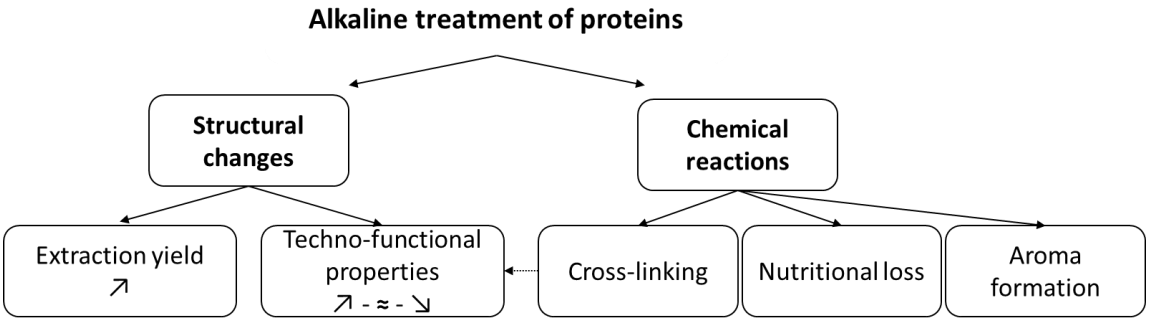
## Abstract

Alkaline conditions have different impacts on proteins. Firstly, they can impact on the protein structure which then influences their solubility and other techno-functional properties. The former generally increases with the difference between pH and pI, and generally increases protein extraction yield. Secondly, chemical reactions can lead to negative nutritional effects by loss of essential amino acids such as lysine. Chemical reactions can also lead to additional crosslinks and to (un)desired color and flavor components. (Pseudo)cereal proteins are often exposed to alkaline conditions e.g. during pretzel production and extraction of rice and pseudo-cereal protein.

## Graphical abstract



## 1. Introduction

Alkaline conditions are used both to extract proteins from cereals and pseudo-cereals and/or to impact the color, flavor and/or texture of their end products (*e.g*. pretzels or alkaline noodles). Alkali increases the protein extraction yield by (1) breaking down the matrix in which the proteins are present and (2) making the protein more soluble. When further from their pI (generally a 4.5 to 5.0 range), proteins carry higher charge. This increases their solubility in aqueous media. High pH can however also induce changes in mostly the tertiary and quaternary structure of proteins and their composition. Changes can also occur during the often performed acidic precipitation afterwards. On top of that, the changes in structure and/or composition, including isomerization, crosslinking and degradation often have techno-functional and nutritional implications.

## 2. Alkali mediated reactions

### 2.1. Chemical reactions

When combining alkaline with heat treatments, several amino acids become involved in chemical reactions. This not only impacts their nutritional value, it also affects the protein complexity as a result of the formation of more and additional types of crosslinks. An overview of the most important reaction mechanisms is shown in Figure 1. The occurrence of cross-links in cereal-based food products has recently been reviewed by Rombouts *et al*. [1] and Lambrecht *et al*. [2]. Under alkaline conditions, disulfide (SS) bond formation through sulfhydryl (SH, $pK_a$ of *ca*. 8.5) oxidation or SH-SS exchange reactions are favored. Also, β-elimination reactions of SS bonds occur which results in dehydroalanine (DHA) and cysteine residues. DHA can further react with cysteine to form lanthionine (LAN) or at pH values exceeding pH 10 also with lysine to form lysinoalanine (LAL) cross-links. Lysine is an essential amino acid which is already limiting in cereals, and especially in wheat gluten [3]. Furthermore, alkaline conditions enhance Maillard reactions which in some cases lead to cross-link formation [1].

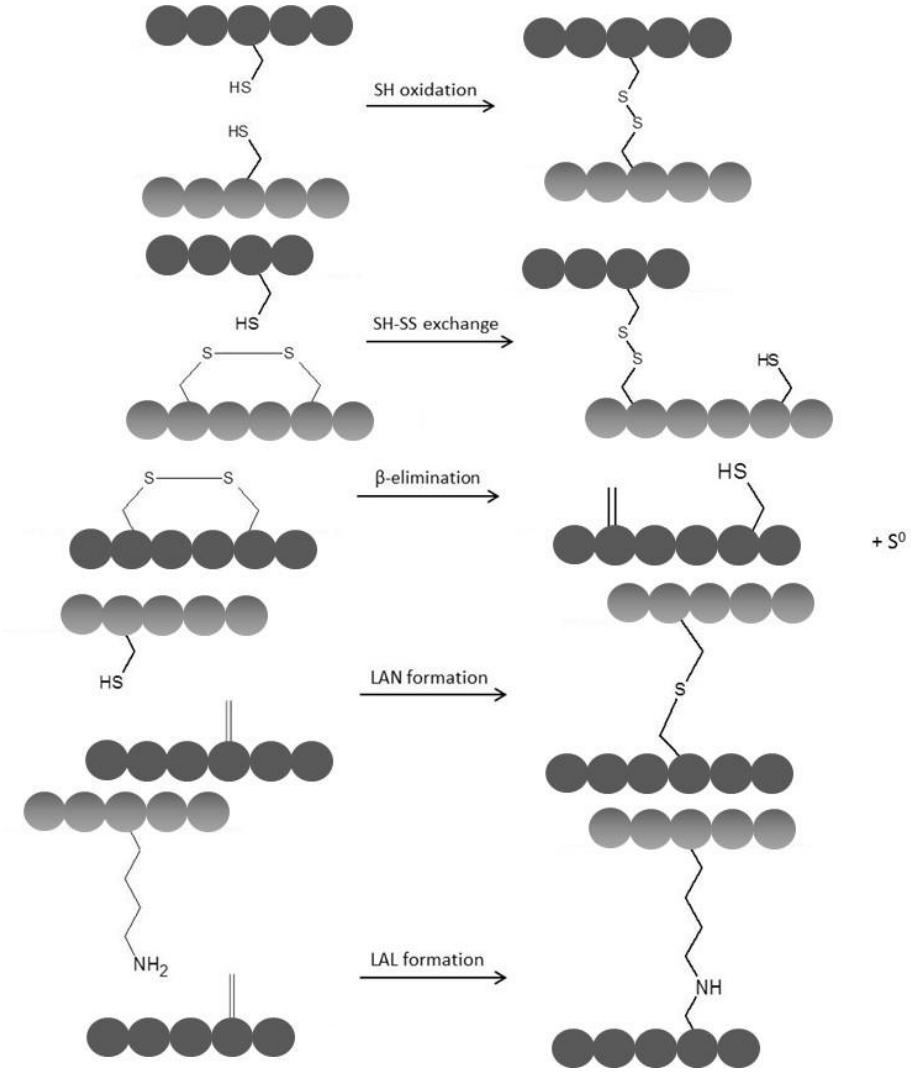



Figure 1: Overview of some common reactions in or between amino acid (●) chains which are enhanced by alkaline conditions. Disulfide (SS) bonds are formed via sulfhydryl (SH) oxidation or SH-SS exchange reactions. β-elimination reactions form SH and dehydroalanine groups which can further react to lanthionine (LAN). Lysinoalanine (LAL) is formed under more severe conditions.

### 2.2. Reactions in model systems

The impact of alkaline conditions on wheat gluten protein model systems has been intensively studied. Wheat gliadins, the monomeric wheat gluten proteins lacking free SH groups, cross-link through SS and DHA-derived cross-links during heating at alkaline pH after β-elimination reaction of intramolecular SS bonds [4]. The rate of the latter increases with pH [5]. Hydrothermal treatment of wheat gluten at pH 8.0 yields to formation of LAN residues while both LAN and LAL residues are formed at pH 13.0 [4]. LAN formation in gliadin is little affected by pH as DHA has a strong tendency to react with SH groups [5]. Once

free SH groups have been released, gliadin polymerizes according to first-order kinetics. The rate of gliadin polymerization increases with temperature and pH [6]. Inclusion of increasing levels of alkaline compounds ($NaHCO_3$, $Na_2CO_3$ or NaOH) during wheat gluten extrusion enhances its polymerization, especially through formation of LAN, and impacts on its hardness, resilience and chewiness [7]. Alkaline treatment of wheat gluten prior to high-temperature compression molding enhances its cross-linking and the flexural strength of the formed bio-plastic. However, molding at high pH also degrades gluten [8].

## 3. Production of protein isolates and its implications

The main storage proteins in most cereals are prolamins (e.g. gliadins and glutenin subunits in wheat, zein in maize, hordein in barley) [9]. Prolamins have in general three conserved regions, with repetitive domains that are differ from species to species. This leads to specific enrichment of certain amino acids (e.g. histidine, glycine, methionine or phenylalanine) [10]. The three conserved regions are shared with 2S albumins, probably via a common ancestral protein of the prolamin superfamily [9]. Unlike prolamins, 2S albumins are soluble in water, but they lack the repetitive domains in between the conserved regions [10]. For oats and rice, the main storage proteins are related to the 11/12S globulins family [10]. As the 2S albumins and 11/12S globulins were identified as the main storage proteins in the pseudocereals quinoa, buckwheat and amaranth [11], it can reasonably be assumed that most pseudo-cereal storage proteins are related to those in cereals but have different solubilities.

### 3.1. Cereal protein

The most common procedure to separate **wheat** (*Triticum aestivum* L.) storage proteins, i.e. gluten, from starch are by dough, dough-batter and batter methods. These rely on the network formation of the gluten proteins (gliadin and glutenin) [12]. However, alkaline extraction can be an option [12] as gluten proteins are highly soluble at high pH [13]. An optimal yield can be obtained around pH 11 [14]. Not much is known about changes of gluten during alkaline extractions, but there is evidence of changes of gluten proteins in other solvents [15, 16]. During alkaline extraction at ambient conditions, the amino acid composition does not change up to pH 11 [14, 17-19]. Yet, little is known about the effect of alkaline extraction on the techno-functionality of gluten. Isolates have emulsifying properties which are comparable to or even better than those of soy isolates [14, 18, 19].

Alkaline extraction of **rice** (*Oryza sativa* L.) protein is commonly used in the rice starch producing industry, and in some cases in combination with surfactants and peptidases [20, 21]. Even up to 97% of the proteins

of milled rice can be extracted under alkaline conditions [22]. As the proteins are organized in protein bodies which are tightly associated with starch and bran tissues, additional mechanical and enzymatic treatments are often also applied [23, 24]. Alkaline extraction can enhance the digestibility and bioavailability of the rice proteins [25, 26]. Nevertheless, also here nutritional and techno-functional hazards are at play [21].

Proteins from **barley** ( *Hordeum vulgare* L.), **rye** (*Secale cereale* L.) and **oat** (*Avena sativa* L.) can also be extracted under alkaline conditions. Alkaline extraction at pH 11 has a strong effect on the secondary structure and techno-functional properties of barley proteins [27]. Rye and oat proteins are well soluble at pH 10.0 (rye) [28] and pH 9.2 (oat) [29]. **Maize** (*Zea mays* L.) proteins, mainly zein, are in practice not extracted in alkali but in alcohol containing media. This is because they contain higher levels of nonpolar than of charged amino acids. Only at pH of at least 11, zein becomes soluble [30].

### 3.2. Pseudo-cereal protein

The interest in pseudo-cereal protein isolates has recently grown [31, 32]. Today, such isolates are almost exclusively obtained by alkaline extraction (pH 8.0-11.0) from defatted meal or flour and subsequent isoelectric precipitation (pH 4.0-5.5) [31]. The storage proteins in pseudo-cereals are related to those in cereals [11, 33] but have different solubility. In contrast to cereal proteins, those of pseudo-cereals such as amaranth, buckwheat or quinoa are mainly albumins and globulins [11]. Pseudo-cereals have a well-balanced amino acid composition and higher biological value than most cereal and legume proteins [34].

The alkaline extraction holds more important risks of nutritional losses for pseudo-cereals than for the already lower nutritional value cereal proteins. Various authors observed a positive linear effect of pH on protein solubility above the isoelectric point for both amaranth [35] and quinoa [36, 37] proteins. Indeed, high protein yield (71-76%) [32, 37] and purity (80-90%) [32, 38, 39] were reported when extracting at pH 11.0. However, Ruiz et al. [40] observed an increased protein yield but decreased protein purity with increasing pH due to non-protein components co-precipitating. It should be noted that most authors studying the impact of alkaline extraction pH on protein yield and structure also included an additional isoelectric precipitation step (pH 4.0-5.5) in their protocols. Föste et al. [41] and Ruiz et al. [40] reported a protein loss of around 20-60% during such precipitation step. On the other hand, Martínez & Añón [39] and Srivastava & Roy [42] observed similar SDS-PAGE profiles after extraction at pH 9.0 and subsequent

precipitation at pH 4.0-6.0, suggesting only a significant decrease in the amount but not in specific protein fractions during the precipitation step.

In spite of the high protein yield and purity obtained with alkaline extraction solvents, their application also leads to extensive protein denaturation. The denaturation temperature ($T_d$) hardly changes with extraction pH as Martínez & Añón reported only a slight decrease of $T_d$ with increasing pH. However, the denaturation enthalpy significantly decreases with increasing extraction pH [38, 39]. At pH values higher than 10.0, the degree of denaturation became so high that an endotherm could no longer be observed for both amaranth and quinoa [38] protein isolates. This is line with an increased degree of molecular dissociation and aggregation observed with size-exclusion high-performance liquid chromatography, a decrease in fluorescence emission intensity in combination with a shift of $\lambda_{max}$ to longer wavelengths, and an increase in the level of SS bonds, free and exposed SH groups for quinoa proteins extracted at pH 11.0 and 12.0 [36]. In a similar way, extraction of amaranth protein at 11.0 leads to formation of large aggregates to a large degree based on hydrophobic interactions since they could be solubilized in sodium dodecyl sulfate containing buffer [39]. Considering this extensive protein denaturation at extreme alkaline pH conditions, most studies on pseudo-cereal protein isolates use mild alkaline extraction conditions (pH 8.0-9.0), followed by precipitation at pH 4.5-5.5 [35, 38, 40, 43-50]. Several authors agree that protein denaturation and structural changes caused by alkaline pH result in modified thermal properties [40, 48], decreased protein solubility [38, 51, 52] and altered emulsifying, foaming [46, 53] and gelation [40, 47] behavior. However, the exact influence of alkaline extraction pH on protein structure-functional relationship remains to be investigated.

## 4. The impact of alkali on cereal-based food production and products

### 4.1. Pretzels

Dipping or spraying extruded wheat dough with *lye*, usually 1.0% sodium hydroxide at about 85 to 93 °C for 10 to 25 seconds, prior to baking and drying results in pretzels with a unique flavor, hard texture and shiny surface [54, 55].The alkali treatment enhances protein hydrolysis, lowers the levels of reducing sugars, dissociates amylose-lipid complexes and gelatinizes starch granules at the dough surface [55]. In addition, it enhances protein network formation mainly through SS bonds and slightly by LAN cross-links [56]. Despite the high impact on flavor and color [55, 56], Maillard reactions cause little protein cross-linking in alkaline-dipped pretzel dough. Baking further increases protein network formation in pretzels.

Next to SS bonds, non-SS cross-links such as LAN and LAL impact the covalent protein network in baked pretzels [1, 56]. Dipping at higher temperatures, longer times or higher concentrations of sodium hydroxide increases protein cross-linking of alkaline-dipped dough and increases the levels of LAN and LAL in baked pretzels [56]. The covalent protein network of pretzels from dough dipped in water contains no LAN or LAL and is less developed than that of pretzel dough dipped in lye [56]. Furthermore, pretzels from water-dipped dough require more force to break than those from alkaline-dipped dough [55].

### 4.2. Alkaline noodles

*Kansui*, usually a mixture of sodium and potassium carbonate, contributes to the flavor, texture and yellow color of alkaline noodles. About 1.0-1.5% *kansui* is used in fresh (*i.e.* uncooked) alkaline noodles resulting in pH values ranging from 9 to 11 depending on the type of alkaline salt and ionic strength used [57]. During noodle production and cooking, these levels of *kansui* enhance intermolecular SS bond formation in sheeted [58] or extruded noodles [59]. Also in other types of noodles low levels of alkaline salts (0.1%-0.3%) can be used as quality improver [57]. While low *kansui* levels enhance SH-SS exchange reactions during cooking, high *kansui* levels additionally enhance LAN and LAL cross-linking [58]. Also in buckwheat containing noodles, the inclusion of alkali salts in the recipe enhances the formation of a more continuous protein network through SS and non-SS cross-links [60]. *Kansui* also increases the firmness and cooking loss of cooked noodles made with wheat [58, 61] or buckwheat [60]. In addition, alkali enhances the rheological properties of both wheat [61] or buckwheat containing fresh noodles [60].

### 4.3. Tortillas

For the preparation of tortillas, maize is treated in 1% $Ca(OH)_2$ at high temperatures (hot lime) for many hours [62]. This process is called nixtamalization and aims to remove the pericarp. Nixtamalization however also affects the protein bodies [63]. The effect on the end product is that the treatment provides a typical 'lime' flavor, aroma and color [64]. Nevertheless, the alkaline treatment also leads to nutritional losses by the formation of LAL [65].

## 5. Perspectives

Alkaline conditions enhance protein unfolding and aggregation. Especially during heating, they favor the formation of SS bonds and DHA-derived cross-links. The consumption of the essential amino acid lysine in LAL formation decreases the nutritional value of protein. Both during extraction of proteins or heating food products, alkaline conditions impact the structure of (pseudo)-cereal proteins and thereby their functionality. In pretzels and noodle production, alkali enhances both covalent protein network formation

and the quality (e.g. taste, color, texture) of the end product. Alkali is very effective at obtaining high extraction yields from pseudo-cereals and cereals. Research on the structure-function relationship of (pseudo-)cereal proteins can enhance the proper use of alkaline salts or alkali-extracted proteins as food ingredients.

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