

Proceeding Paper

# Insights on the Interaction between Kefiran and Whey Proteins through Computational Analyses <sup>†</sup>

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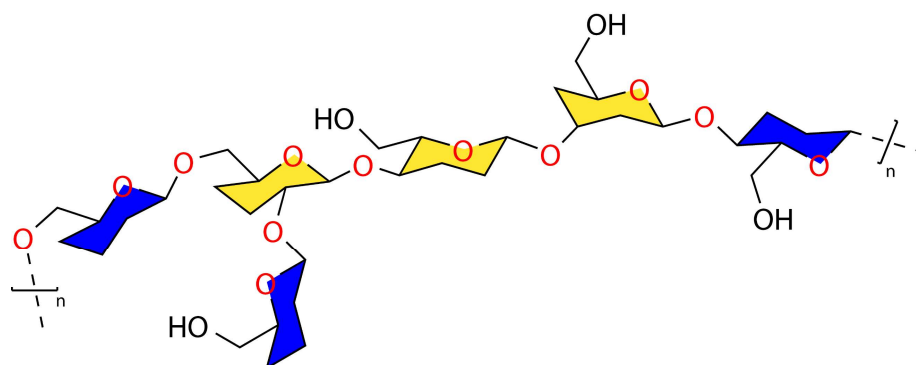
<sup>†</sup> Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: <https://ecsoc-27.sciforum.net/>.

**Abstract:** Kefiran is an exopolysaccharide produced by milk fermentation that is widely used in the food industry mainly to improve the rheological properties of foods, as well as for the formation of biofilms. Most of these properties are related to its interaction with milk proteins. It has been reported that kefir has a molecular weight of up to 10<sup>7</sup> Da and that it is formed by glucose and galactose molecules in equimolar quantities; however, its folding form is not completely understood. In this work it was elucidated that a model of the 20 kDa kefir folds in a helical shape. In addition, it was determined that whey proteins can form complexes with kefir.

**Keywords:** kefir; molecular docking; whey proteins; kefir granules

## 1. Introduction

Kefiran is an exopolysaccharide (EPS) produced during the lactic-alcoholic fermentation of milk with kefir granules, which are a complex matrix that behaves like a symbiont, formed by bacteria (lactic and acetic acid) and yeast [1]. It is a heteropolysaccharide consisting of subunits of D-glucopyranose and D-galactopyranose in equimolar amounts [2,3] with a molecular weight (MW) ranging between 55 and 10,000 kDa, depending on extraction and purification conditions [4,5]. Figure 1 shows the chemical structure of a subunit of kefir proposed by Micheli et al. [4], which has an approximate MW of 1 kDa. Due to the  $\beta$ -glycosides, like cellulose, it has been reported that it cannot be hydrolyzed by the enzymes of the digestive tract, giving it a prebiotic character [5–7].



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**Figure 1.** Chemical structure of the kefiran repeating subunit [4]. D-glucose (blue) and D-galactose (yellow).  $n = [55-10,000]$  (Backbone  $[\rightarrow 6)\beta\text{DGlc}p(1\rightarrow 2,6)\beta\text{DGal}p(1\rightarrow 4)\alpha\text{DGal}p(1\rightarrow 3)\beta\text{DGal}p(1\rightarrow 4)\beta\text{DGlc}p(1\rightarrow ]$ , with a branched  $[\beta\text{DGlc}p(1\rightarrow 2)]$  to the first  $\beta\text{DGal}p$  residue).

The EPS is produced mainly by *Lactobacillus* lactic acid bacteria (LAB) including *Lactobacillus kefiranofaciens*, *Lactobacillus kefirgranum*, *Lactobacillus parakefir*, *Lactobacillus kefir* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [7], although it has been seen that producing this EPS with by an isolated strain, rather than the complete microbial consortium, does not generate a polymer with the same characteristics as that present in kefir [8]. This suggests a complex interplay between the various bacteria, and potentially a co-operative biosynthesis. Kefiran interactions with milk proteins has been reported to improve the rheological properties of milk derivative products [7]. Some of the best known applications of this polysaccharide are as an emulsifying agent [9], stabilizer thickener, or gelling agent [10]. It has also been reported to have antioxidant [11], antimicrobial, [12] and even hypotensive activity [13]. Additionally, due to their resistance to digestive hydrolysis, kefirans can be used as vehicles for the protected delivery of probiotic bacteria and even drugs [5].

As part of our work to characterize the interaction of EPS with mycotoxins in milk, we want to present preliminary results where we have modelled a sub portion of the kefiran structure and evaluated the interaction of this saccharide with two of the main lactic proteins:  $\alpha$ -lactalbumin (ALA) and  $\beta$ -lactoglobulin (BLG). This will allow us to access a broader perspective of the possible interference these proteins can cause in the interactions between kefiran and the mycotoxins.

## 2. Materials and Methods

### 2.1. Design and Geometric Optimization of Kefiran

Starting from 20 subunits of kefiran ( $\approx 20$  kDa) [6], the 3D model was built using the programs *ChemDraw* and *Chem3D* (22.2 version); this was optimized by energy minimizations using the MM2 force field with a maximum of 10,000 iterations.

### 2.2. Molecular Docking

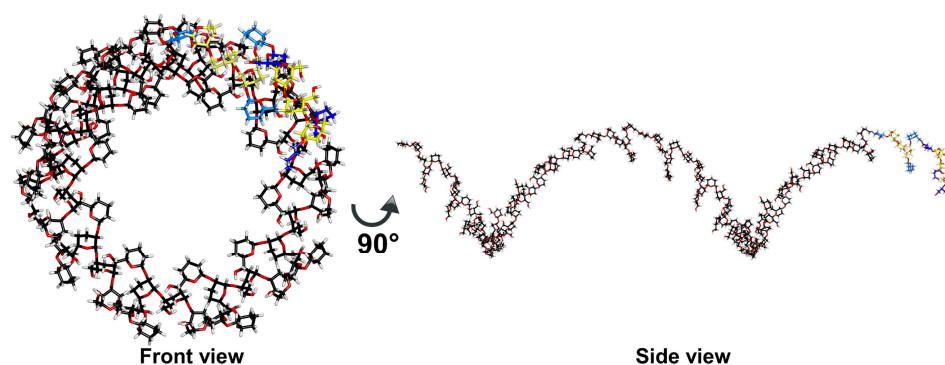
Prior to the evaluation of the molecular docking (MDock), the structural models of the whey proteins obtained from the Protein Data Bank: ALA (1HFZ) and BLG (1BEB), were corrected by the addition of missing hydrogens according to the pKa of the titratable amino acids at pH 6.8 (pH of milk for EPS interaction assays with mycotoxins), using the *ProteinPrepare* tool on the *PlayMolecule* server [14].

MDock evaluations were performed on the SwissDock server [15] by the “Accurate with certain limitations” docking protocol. As this program performs dockings that are based on the interaction with small molecules (maximum 250 atoms). Through the *program BIOVIA Discovery Studio Visualizer v20.1.0.19295* the optimized model (Section 2.2) was cut into two subunits (226 atoms each) to perform the evaluation of their interactions with the previously protonated whey proteins.

## 3. Results and Discussion

### 3.1. Design and Geometric Optimization of the Kefiran Model

According to the optimization of the 20 subunits of kefiran structure, it was observed that it tends to fold into an  $\alpha$ -helix-like conformation (Figure 2). This result is in agreement with Exarhopoulos et al. [16], as they reported that kefiran solubilized in aqueous solution adopts a helix.



**Figure 2.** Front and side views of the 3D model of 20-subunit kefir. D-glucose (blue) and D-galactose (yellow).

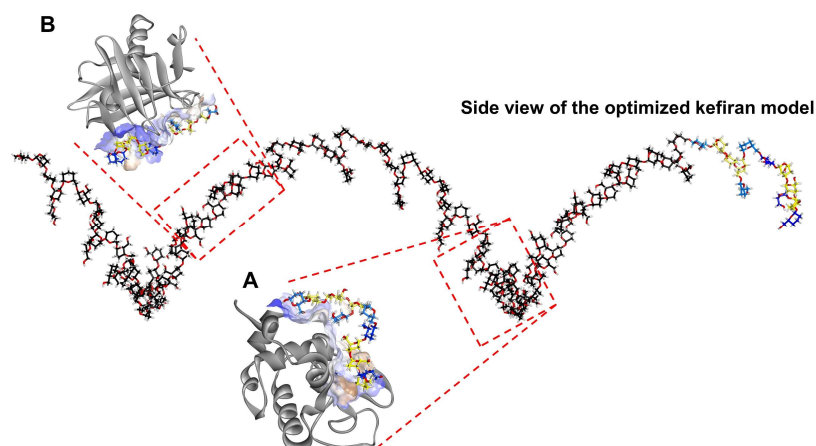
### 3.2. Molecular Docking

According to the free binding energies ( $\Delta G_b$ ) obtained from MDock evaluations (Table 1), kefir can form complexes with both proteins. These data showed that they have a similar affinity for EPS. In addition, it was observed that the interactions occur in the hydrophobic regions of the protein (Figure 3). Something similar happened when the interaction between an EPS produced by *Streptococcus thermophilus* LY03 and BLG was evaluated, where MDock showed that the formation of the complexes depends not only on hydrogen bonds, but also on hydrophobic interactions [17]. With these results and taking as reference the model on Figure 2 (Section 3.1), it is considered that the way in which the two proteins participate in the interaction with EPS is that of the ALA binding the region where the chain bend is formed (Figure 3A), while the BLG interacts in the linear regions (Figure 3B). Therefore, if a greater number of protein units were considered, it is likely that they would surround the EPS, creating the conditions for enhanced interactions between proteins and the polysaccharide to allow the formation of more extensive networks. This phenomenon would result in an increase in rheological properties, such as viscosity. However, the fact that more EPS and proteins bind should create interference in the interactions between mycotoxins and kefir.

**Table 1.** Free binding energy of formed complexes between the two subunits of kefir with whey proteins.

Complex	$\Delta G_b$ (kcal/mol)
ALA-kefir	-10.52
BLG-kefir	-11.01

ALA:  $\alpha$ -lactalbumin; BLG:  $\beta$ -lactoglobulin;  $\Delta G_b$ : Binding free energy.



**Figure 3.** Side view of the 3D model of 20-subunits kefiran. (A) Complex formed between 2 subunits of kefiran with ALA. (B) Complex formed between 2 subunits of kefiran with BLG. D-glucose (blue) and D-galactose (yellow).

The proposed regions in the kefiran structure where ALA and BLG bind are based on the work done by Ayala-Hernandez et al. [18] who evaluated the interaction of milk proteins with an EPS produced by *Lactococcus lactis* ssp. *cremoris* by scanning electron microscopy. They found that the proteins interact wrapping the filament-shaped EPS, whose structure they attribute to the dehydration process suffered by the samples. In addition, they observed that proteins began to aggregate around the EPS which allowed the formation of a biofilm.

#### 4. Conclusions

According to the results obtained, it was possible to identify that kefiran folds helically, which allows it to have a larger surface to interact with whey proteins. Likewise, it was determined that the complexes formed between ALA and BLG with EPS are mainly due to hydrophobic interactions.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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