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SCIENTIFIC REPORT

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Phase I

Objective 1: Testing the affinity of whey proteins for carotenoid and anthocyanins using in silico techniques

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Chapter 1. The main structural characteristics of proteins whey

1.1. General aspects

The term of whey proteins is attributed to those proteins that remain in the aqueous phase of milk after precipitation and separation of caseins at pH 4.6 and 20°C. In this class are included: β-lactoglobulin (β-LG), α-lactalbumin (α-LA), serum albumin (SAB), immunoglobulins (Ig) and lactoferrin (LF). β-LG and α-LA are further characterized for structural and conformational features, as are the main whey proteins.

1.2. β-lactoglobulin

β-LG is the major whey protein that it can be found in cow's milk in a concentration of 2.4 g/L (de la Fuente, 2002). Characterizations of protein by composition in amino acid, polypeptide sequence and isoelectric point have revealed the existence of a genetic polymorphism. Ten genetic variants of bovine β-LG have been identified, seven of which being isolated and characterized. All genetic variants of β-LG contain 162 amino acid residues, the difference appearing in one or three positions. Although genetic variants A and B differ only in two positions, the substitution of Gly with Asp in position 4 is especially important because it increases the ability of self-association of variant A. This phenomenon can be explained by the formation of additional salt bridges between the remaining carboxylic group of Asp and any other basic group.

1.2.1. The primary structure of β-LG

The amino acid composition of the reference protein is Asp_{10}, Asn_5, Thr_8, Ser_7, Glu_{16}, Gln_9, Pro_8, Gly_4, Ala_{15}, Cys_5, Val_9, Met_4, Ile_{10}, Leu_{22}, Tyr_4, Phe_4, Lys_{15}, His_2, Trp_2 and Arg_3, with a molecular weight from 18,277 Da. It can be seen that β-LG contains a large number of essential amino acids. However, their bioavailability is low due to the resistance of the protein to proteolysis in the acidic pH range (Reddy et al. 1988).

1.2.2. The secondary structure of β-LG

Monaco et al. (1987) have described the molecule as a spherical shape having a diameter of 2.5 Å, consisting of a short portion of the α-helix and eight antiparallel polypeptide chains with β-sheet folded structure. The eight antiparallel chains are represented by residues 15 to 27 (A), 35-44 (B) 49-56 (C) 65-74 (D), 82-85 (E), 91-97 (F), 102-111 (G), and 116-124 (H). The fragment 131-140 has α-helix structure, whereas fragments 28-31, 45-48, 61-64, 98-101 and 112-115 presents reverse β-sheet structures, while segments 74-82 and 125-130 are rings with structure not well defined.

1.2.3. Conformational flexibility of β-LG

At 25°C and pH range of 4.0 to 6.5, bovine β-LG is a dimer. When the pH value changes close to 7.0, the molecule undergoes a number of conformational transitions characterized by increasing of reactivity of the carboxyl groups and SH^{121} group, which under physiological conditions is located within the molecule. Phillips et al. (1994) studied the conformational changes that occur when dimer dissociates at pH

7.5 (so-called Tanford transition). It has been suggested that these structural changes affect the position were β-LG bind the retinol, thus influencing the biological function of the protein.

1.3. α-lactalbumin

α-LA is a small protein, with a molecular mass of 14,174 Da, pHi 4.0-6.0, which is able to bind calcium ions. First, α-LA fulfills an important function in secretory cells breast, being one of the two components of lactose synthase, the enzyme that catalyze the final stage of lactose biosynthesis. Secondly, α-LA has one binding site for Ca^{2+}, being used as a model system for study the effects of calcium ion binding to proteins, peptides, membranes, etc. Thirdly, α-LA can form a series of partially unfolded states, being used as classic molten molecules used to elucidate the mechanisms of protein folding/unfolding.

1.3.1. Primary, secondary and tertiary structures of α-LA

The protein contains 123 amino acid residues with two major genetic variants (A and B). Variant B is present in milk from Bos taurus, whereas variant A is present in milk from Bos indicus species. Variant A contains Glu in position 10, while in variant B Glu is substituted by Arg (Stănciuc, 2009). The primary structure of α-LA variant B contains: Ala_3, Arg_1, Asn_8, Asp_{13}, Cys_8, Gln_8, Glu_7, Gly_6, His_3, Ile_8, Leu_{13}, Lys_{12}, Met_1, Phe_4, Pro_2, Ser_7, Thr_7, Trp_4, Tyr_4, and Val_6. α-LA has a high content of essential amino acids (Trp, Phe, Tyr, Leu, Ile, Thr, Met, Cys, Lys, and Val), representing 63.2% of the protein total amino acids and 51.4% of the essential amino acids from milk proteins. The domain with helical structure contains three α-helix, which are stable at pH variation (fragments 5-11, 23-34 and 86-98), a pH dependent fragment (105-100) and three portions with small helix structure (18-20, 3 and 115-118). The flexible fragment (105-110) presents a helix configuration at pH ranging from 6.5 to 8.0 (N'Negue et al., 2006). The region with β-structure consists of three antiparallel chains (fragments 41-44, 47-50 and 55-56) and a short chain with helix structure (77-80). These two domains are stabilized by disulfide bond between residues Cys at positions 73 and 91, and to a lesser extent by the disulfide bond Cys^{61} - Cys^{77}. Overall, the structure is stabilized by four disulfide bonds (Cys^{6} - Cys^{120}, Cys^{61} - Cys^{77}, Cys^{73} - Cys^{91} and Cys^{28} - Cys^{111}).

1.3.2. Conformational flexibility of α-LA

The binding of cations increases the stability of the protein conformation. The same effect has binding ions Mg^{2+}, Na^{+} and K^{+}. The binding of Zn^{2+} cause destabilization of the molecule resulting in aggregation of the molecules and increase in susceptibility to proteolysis. In the presence of high concentrations of Zn^{2+}, α-LA partially unfolds and aggregates. In the absence of calcium ions, but in the presence of physiological concentration of Mg^{2+}, Na^{+} and K^{+}, the transition temperature varies in the temperature range of 30-45°C.

Chapter 2. Structural features of carotenoids and polyphenols

2.1. Carotenoids

Chemically, carotenoids are tetraterpenes and their structures are based on 40-carbon polyene chain with conjugated double bonds (3–13) along this chain. Though they seem to be hidden, carotenoids are one of the most wide spread and ubiquitous lipid soluble pigments in nature (e.g. in leaves, fruits, flowers, teguments, etc.) and so far, more than 750 naturally occurring carotenoids have been identified (Britton et al., 2008). Carotenoids are produced by all photosynthetic organisms, by fungi and by non-photosynthetic bacteria and conversely, they are required in the diet of animals as antioxidants or vitamins, but also to produce their tissues pigmentation, as is the case of the feathers of birds or the exoskeleton of crustaceans.

Remarkably, carotenoid compounds only differ in the following chemical characteristics, which give rise to the different carotenoids structures: (i) the presence and number of oxygen atoms in the molecule (oxygenated carotenoids are xanthophyll and non-oxygenated are carotenes), (ii) the hydrogenation of the carbon polyene chain, (ii) the cyclization at one/both ends of the molecule, usually with a e-ionone or b-ionone rings and the (iv) length of the chromophore (Meléndez-Martínez et al., 2007).

Carotenoids can be divided into two main groups, depending on the functional groups:

- ✓ xanthophylls, which contain oxygen as functional groups, such as lutein and zeaxanthin;
- ✓ carotene, with a hydrocarbonic chain, containing no functional groups, such as α-carotene, β-carotene and lycopene.

Fruits and vegetables are the main sources of carotenoids and play an essential role in the diet through vitamin A activity (Haskell, 2013). In addition to the role of vitamin A, they have antioxidant activity, provides intercellular communication and specific activity of the immune system (Skibsted, 2012; Stephensen, 2013). Epidemiological studies have shown that a diet rich in carotenoids ensures a lower incidence of cancer, cardiovascular disease, aging and cataract (Meyers et al., 2014; Sharon et al., 2012). Carotenoids deficit results in clinical conjunctivitis and corneal disorders, including dry eye, night blindness, keratomalacia, corneal ulceration, cicatrices and irreversible blindness (Sommer, 2008). Deficiencies in provitamin A lead to visual disability and increased mortality due to immunity innate and low adaptive (Stephensen, 2001). Lycopene shows the highest antioxidant activity. This feature recommends lycopene in protecting cellular systems against reactive oxygen and nitrogen reactive species, thus preventing cardiovascular disease (Müller et al., 2015).

2.2. Polyphenols

Polyphenolic compounds are of major interest for the food, pharmaceutical and medicine due to their beneficial effects on health, in particular in the treatment and cancer prevention (Chen et al., 2011), cardiovascular disease (Kuriyama et al., 2006; Mursu and et al., 2008), anticarcinogenic effects (Jeong et al., 2011; Ogunleye et al., 2009), ulcer (Zakaria et al., 2011), antithrombotic (Han et al., 2012; Tao et al., 2012), anti-inflammatory (Bear et al., 2012; Zimmer et al., 2012), antiallergenic (Chung and Champagne,

2009; Schmitz-Eiberger and Blanke, 2012), anticoagulants (Bijak et al., 2011), immunomodulators (Schütz et al., 2010) and antimicrobial (Silva et al., 2012; Xia et al. 2011), vasodilators and analgesics (Santoz et al., 2010). Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contains up to 200–300 mg polyphenols per 100 grams fresh weight.

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans (Spencer et al., 2008).

Chapter 3. Investigations on the binding mechanism of compounds from vegetable extracts to β-lactoglobulin and α-lactalbumin and analysis of relevant atomic particularities for assessing stability of complexes

3.1. Introduction

Whey proteins have been extensively studied in terms of binding ability of hydrophobic ligands, such as fatty acids and vitamins, raising a number of advantages, as follows: increase the absorption of fatty acids (Perez et al. 1992), modify the kinetics of enzymatic hydrolysis of the protein (Mandalari et al. 2009), protection of ligands against oxidation or other stress factors and modifying the bioavailability of ligands (Riihimäki-Lampen, 2009). In food products, the binding properties and therefore the biological properties of the complexes can be affected by the structure of proteins and/or the presence of other proteins able to compete for binding sites. For example, the ability of β-LG to bind many hydrophobic compounds, such as retinol, vitamin D, cholesterol, curcumin acids and their derivatives, protoporphyrin IX, aromatic compounds and cations catechin has been demonstrated (Liskov et al. 2011; Le Maux et al. 2012; Puyol et al. 1994; Sneharani et al. 2010; Kanakis et al. 2011; Dufour et al. 1990, 1992; Liu et al. 2011).

α-LA ability to bind oleic acid with the formation of HAMLET/BAMLET (Human/Bovine α-lactalbumin made lethal to tumor cells) is already known for about 15 years (Svensson et al., 2000). This complex has a cytotoxic activity for cancer cell lines, whereas healthy cells are not affected. α-LA can bind other compounds such as resveratrol (Hemar et al., 2011), sodium oleate (Kehoe and Brodkorb, 2014), genistein, kaempferol (Moeen and Mohammadi, 2015) etc.

3.2. Analysis of α-lactalbumin and β-lactoglobulin structure by in silico methods

In order to evaluate the binding mechanism and affinity of biologically active compounds, α-LA and β-LG molecules models were taken from the RCSB Protein Data Bank (www.rcsb.org). Studies at single molecule level were made using the protein models 1F6S.pdb (one monomer of bovine α-LA it was considered; Chrysina et al., 2000) and 3NPO.pdb (bovine β-LG; Loch et al., 2011) solved by X-ray crystallography. These models showed very good resolution and high stability.

Specific structural bioinformatics methods were initially applied for advanced characterization of whey proteins. Based on the physicochemical properties of amino acids the following parameters were analyzed by using ProtParam: molecular weight, isoelectric point, atomic composition, aliphatic and hydrophilicity index (GRAVY), which for a peptide or protein is calculated as the sum of the hydrophobicity index values for all amino acids divided by the number of residues in the sequence. Surface properties of α-LA and β-LG were assessed using the program GETARIA.

For an advanced characterization of the models, α-LA and β-LG molecules were first refined by removing all non-protein compounds. Geometry optimization of molecules was then conducted in vacuum; minimization of the potential energy was performed such as to ensure the removal of any possible geometric distortion and repulsive interactions between atoms.

Such optimized molecules were placed in the center of a parallelepidic reaction box, having suitable size for the molecular system, which were subsequently filled with single point charged (SPC) water molecules (explicit solvent). By solvation, it ensures a higher degree of native protein folding due to the involvement of the hydrogen bonds (Mogilner et al., 2002). Solvated molecular systems were then optimized by successively using committed algorithms, Steepest Descent and limited-memory Broyden-Fletcher-Goldfarb-Shanno, as in the case optimization in vacuum.

In order to identify specific characteristics of the protein molecules at various temperatures, the systems were heated at 25°C and 80°C using a Berendsen thermostat, followed by the systems equilibration at indicated temperatures.

The molecular mechanics and molecular dynamics simulations were performed using the software Gromacs (v. 4.5.5.), which runs with Linux operating environment, using the Gromos96 43a1 or AMBER (Assisted Model Building and Energy Refinement) force fields under parallelization conditions, on a computer equipped with processor Intel(R)Core(TM)2CPU1.86GHz6300. For structural and conformational analysis, specific PDBsum instruments (Laskowski, 2009) and Visual Molecular Dynamics (VMD) were used (Humphrey et al., 1996).

3.3. Studies on the molecules interactions

For interfacing the models (α-LA with β-carotene and β-LG with cyanidin 3-O rutinoside), the PatchDock server was used (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005), that provides the molecular docking on the basis of shape complementarity, generating 100 different complexes, which are then prioritized by scoring function. PatchDock provides rigid interfacing of molecules, resulting models that are then refined using FireDock server (Andrusier et al., 2007).

The refinement of ten best models was based on the relative orientation of the specific groups/atoms at the interface between the two molecules in the complex. Then, a new classification of the models was performed, mainly taking into account the interaction energy between molecules, and some others factors such as energetic ones (Andrusier et al., 2007). In order to assess the total number of ligand binding sites in the protein molecule and to evaluate the particularities of interaction between the two molecules within each investigated complex, the ten best results obtained in computational models were considered following docking and refinement methods. The analysis of the resulted complex was done using dedicated software LigPlot+v1.4.5. (Laskowski and Swindells, 2011) and PDBePISA (Krissinel and Henrick, 2007; Krissinel, 2009).

3.4. α-lactalbumin-β-caroten complex

In order to study the mechanism of β-carotene binding to α-LA, the atomic particularities of all refined docking solutions were analyzed. Four binding sites for β-carotene were observed on the surface of α-LA. The main binding site involves the following amino acids: His^{32}, Thr^{33}, Asn^{44}, Asn^{45}, Asp^{46}, Ser^{47}, Glu^{49}, Asn^{56}, Lys^{58}, Tyr^{103}, Trp^{104} and Leu^{105}. The second binding site involves the following amino acids: Cys^{28}, Phe^{31}, His^{32}, Lys^{108}, Ala^{109}, Leu^{110}, Ser^{112}, Glu^{113}, Lys^{114}, Gln^{117} and Trp^{118}. The third binding site involves the common amino acids Gln^{2}, Lys^{5}, Gln^{117} and Trp^{118} and is characterized by low levels of contact

surfaces and high binding energy, indicating a low affinity of molecules. A low affinity was also found for the fourth binding site of β-carotene to α-LA surface, with the participation of the following amino acids: Asn^{74}, Ile^{75}, Ser^{76}, Asp^{78}, Lys79, Asp^{87} and Met^{90}.

3.5. β-lactoglobulin - cyanidin-3-O-rutinosid complex

A similar approach was considered in evaluating the atomic particularities regarding the binding of cianidin-3-O-rutinosid to β-LG and the affinity between these two molecules. Two different binding sites were defined on the surface of β-LG molecule. The primary binding site for cyanidin 3-O rutinozid involves the following amino acids Arg^{10}, Glu^{11}, Lys^{13}, Asp^{14}, Asp^{84} and Leu^{85}, whereas the second one consists of Gln^{43}, Asn^{44}, Asn^{45}, Thr^{48}, Tyr^{50}, Gln^{65}, Asn^{66}, Pro^{67} and His^{68}.

A detailed analysis at atomic level indicates that heat treatment lead only to minor conformational changes at protein surface, which includes the sites of interaction with the ligand.

Selective references:

Andrusier, N., Nussinov, R., Wolfson, H. J. (2007). FireDock: fast interaction refinement in molecular docking. Proteins: Structure, Function, and Bioinformatics, 69(1), 139-159.

Bear, I. N., Lesjak, M. M., Orcic, D. Z., Simin, N. D., Cetoyevic-Simin, D. D., Bozin, B., și colab. (2012). Comparative analysis of phenolic profile, antioxidant, anti-inflammatory and cytotoxic activity of two closely-related Plantain species: Plantago altissima L. and Plantago lanceolata L. LWT — Food Science and Technology, 47, 64–70.

Britton, G. (2008). Functions of intact carotenoids. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), Carotenoids (pp. 189–212). Basel: Birkhäuser.

Chen, D., Wan, S. B., Yang, H., Yuan, J., Chan, T. H., Dou, Q. P. (2011). EGCG, green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment. Advances in Clinical Chemistry, 53, 155–177.

Chung, S. Y., Champagne, E. T. (2009). Reducing the allergenic capacity of peanut extracts and liquid peanut butter by phenolic compounds. Food Chemistry, 115, 1345–1349.

Chrysina, E. D., Brew, K., Acharya, K. R. (2000). Crystal structures of apo-and holo-bovine α- lactalbumin at 2.2-Å resolution reveal an effect of calcium on inter-lobe interactions. Journal of Biological Chemistry, 275(47), 37021-37029.

De la Fuente, M., Singh, H., Hemar, Y. (2002). Recent advanced in the characterisation of heat-induced aggregates and intermediates of whey proteins, Trends in Food Science and Technology, 13, 262-274.

Dufour, E., Roger, P., Haertlé, T. (1992). Binding of benzo(α)pyrene, ellipticine, and cis-parinaric acid to betalactoglobulin: influence of protein modifications. Journal of Protein Chemistry, 11(6), 645-652.

Duhovny, D., Nussinov, R., Wolfson, H. J. (2002). Efficient unbound docking of rigid molecules. In Algorithms in bioinformatics (pp. 185-200). Springer Berlin Heidelberg.

Han, N., Gu, Y., Ye, C., Cao, Y., Liu, Z., Yin, J. (2012). Antithrombotic activity of fractions and components obtained from raspberry leaves (Rubus chingii). Food Chemistry, 132, 181–185.

Haskell, M. J. (2013). Provitamin A carotenoids as a dietary source of vitamin A.In S. A. Tanumihardjo (Ed.), Carotenoids and human health (pp. 249–260). Humana Press.

Humphrey, W., Dalke, A., Schulten, K. (1996). VMD: visual molecular dynamics. Journal of Molecular Graphics, 14(1), 33-38.

Jeong, W. Y., Jin, J. S., Cho, Y. A., Lee, J. H., Park, S., Jeong, S. W., și colab. (2011). Determination of polyphenols in three Capsicum annuum L. (bell pepper) varieties using high-performance liquid chromatography–tandem mass spectrometry: their contribution to overall antioxidant and anticancer activity. Journal of Separation Science, 34, 2967–2974.

Kehoe, J.J., Brodkorb, A. (2014). Interactions between sodium oleate and α-lactalbumin: The effect of temperature and concentration on complex formation. Food Hydrocolloids 34, 217-226.

Krissinel, E., Henrick, K. (2007). Inference of macromolecular assemblies from crystalline state. Journal of molecular biology, 372(3), 774-797.

Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., și colab., (2006). Green tea consumption and mortality due to cardiovascular disease, cancer and all causes in Japan. Journal of the American Medical Association, 296, 1255–1265.

Laskowski, R. A. (2009). PDBsum new things. Nucleic Acids Research, 37(suppl 1), D355-D359.

Laskowski, R. A., Swindells, M. B. (2011). LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. Journal of Chemical Information and Modeling, 51(10), 2778-2786.

Liu, L., Kitova, E.N., Klassen, J.S. (2011). Quantifying protein-fatty acid interactions using electrospray ionization mass spectrometry. Journal of the American Society for Mass Spectrometry 22(2), 310–318.

Loch, J. I., Bonarek, P., Polit, A., Riès, D., Dziedzicka-Wasylewska, M., Lewiński, K. (2013). Binding of 18-carbon unsaturated fatty acids to bovine β-lactoglobulin—structural and thermodynamic studies. International Journal of Biological Macromolecules, 57, 226-231.

Mandalari, G., Mackie, A.M., Rigby, N.M., Wickham, M.S.J., Mills, E.N. (2009). Physiological phosphatidylcholine protects bovine beta-lactoglobulin from simulated gastrointestinal proteolysis. Molecular Nutrition & Food Research, 53(S1), S131–S139.

Meyers, K. J., Mares, J. A., Igo, R. P., Truitt, B., Liu, Z., Millen, A. E., și colab. (2014). Genetic evidence for role of carotenoids in age-related macular degeneration in the carotenoids in age-related eye disease study (CAREDS). Investigative Ophthalmology & Visual Science, 55(1), 587–599.

Mogilner, I. G., Ruderman, G., Grigera, J. R. (2002). Collagen stability, hydration and native state. Journal of molecular Graphics and Modelling, 21(3), 209-213.

Mohammadi F., Moeeni, M. (2015). Analysis of binding interaction of genistein and kaempferol with bovine α-lactalbumin. Journal of functional foods, 12, 458–467.

Mursu, J., Voutilainen, S., Nurmi, T., Tuomainen, T. P., Kurt, S., Salonen, J. T. (2008). Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-ages Finnish men. Journal of Nutrition, 100, 890–895.

Müller, L., Caris-veyrat, C., Lowe, G., Böhm, V. (2015). Lycopene and its antioxidant role in the prevention of cardiovascular diseases — A critical review. Critical Reviews in Food Science and Nutrition, http://dx.doi.org/10.1080/10408398.2013.801827.

N'Negue, M.-A, Miclo, L., Girardet, J.-M., Campagna, S., Molle, D., Gaillard, J.-L. (2006). Proteolysis of bovine α-lactalbumin by thermolysin during thermal denaturation, International Dairy Journal, 16, 1157–1167.

Ogunleye, A. A., Xue, F., Michels, K. B., (2009). Green tea consumption and breast cancer risk or recurrence: A meta-analysis. Breast Cancer Research and Treatment, 119, 477–484.

Pérez-Gálvez, A., Mínguez-Mosquera, M. I. (2005). Esterification of xanthophylls and its effect on chemical behavior and bioavailability of carotenoids in the human. Nutrition Research, 25(7), 631– 640. http://dx.doi.org/10.1016/j.nutres.2005.07.002.

Perez, M.D., Sanchez, L., Aranda, P., Ena, J., Oria, R., Calvo, M. (1992). Effect of beta-lactoglobulin on the activity of pregastric lipase. A possible role for this protein in ruminant milk. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 1123(2), 151-155.

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Puyol, P., Perez, M.D., Peiro, J.M., Calvo, M. (1994). Effect of binding of retinol and palmitic acid to bovine betalactoglobulin on its resistance to thermal denaturation. Journal of Dairy Science, 77(6), 1494-1502.

Reddy M., Kella N.K.D., Kinsella J.E. (1988). Structural and conformational basis of the resistance of β-lactoglobulin to peptic and chymotryptic digestion, Journal of Agriculture and Food Chemistry, 36, 737-741;

Riihimäki-Lampén, L. (2009). Interactions of natural products with beta-lactoglobulins, members of the lipocalin family. University of Helsinki

Santoz, M. D., Almeida, M. C., Lopez, N. P., Souza, G. E. P. (2010). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenols CGA. Biological and Pharmaceutical Bulletin, 29, 2236–2240.

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., Wolfson, H. J. (2005). PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic acids research, 33(suppl 2), W363-W367.

Schütz, K., Saß, M., With, A., Graubaum, H. J., Grünwald, J., (2010). Immune-modulating efficacy of a polyphenol-rich beverage on symptoms associated with the common cold: a double-blind, randomised, placebo-controlled, multi-centric clinical study. British Journal of Nutrition, 104, 1156–1164.

Sharoni, Y., Linnewiel-Hermoni, K., Khanin, M., Salman, H., Veprik, A., Danilenko, M., și colab. (2012). Carotenoids and apocarotenoids in cellular signaling related to cancer: A review. Molecular Nutrition & Food Research, 56(2), 259–269.

Skibsted, L. H. (2012). Carotenoids in antioxidant networks colorants or radical scavengers. Journal of Agricultural and Food Chemistry, 60(10), 2409–2417.

Silva, J. C., Rodrigues, S., Feas, X., Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. Food and Chemical Toxicology, 50, 1790–1795.

Sneharani, A.H., Karakkat, J.V., Singh, S.A., Rao, A.G.A. (2010). Interaction of curcumin with beta-lactoglobulin—Stability, spectroscopic analysis, and molecular modeling of the complex. Journal of Agricultural and Food Chemistry, 58(20), 11130-11139.

Sommer, A. (2008). Vitamin A deficiency and clinical disease: A historical overview. The Journal of Nutrition, 138(10), 1835–1839.

Stănciuc, N. (2009). Proteinele laptelui, Relație structură-funcție. Editura Academica, Galati.

Stephensen, C. B. (2001). Vitamin A, infection, and immune function. Annual Review of Nutrition, 21(1), 167–192. http://dx.doi.org/10.1146/annurev.nutr.21.1.167.

Stephensen, C. B. (2013). Provitamin A carotenoids and immune function.In S. A. Tanumihardjo (Ed.), Carotenoids and Human Health (pp. 261–270). Humana Press.

Svensson, M., Hakansson, A., Mossberg, A. K., Linse, S., Svanborg, C. (2000). Conversion of α- lactalbumin to a protein inducing apoptosis. Proceedings of the National Academy of Sciences of the United States of America, 97(8), 4221-4226.

Tao, W. W., Duan, J. A., Yang, N. Y., Tang, Y. P., Liu, M. Z., Qian, Y. F. (2012). Antithrombotic phenolic compounds from Glycyrrhiza uralensis. Fitoterapia, 83, 422–425.

Xia, D., Wu, X., Shi, J., Yang, Q., & Zhang, Y. (2011). Phenolic compounds from the edible seeds extract of Chinese Mei (Prunus mume Sieb. Et Zucc) and their antimicrobial activity. LWT — Food Science and Technology, 44, 347–349.

Zakaria, Z. A., Hisam, E. E. A., Rofiee, M. S., Norhafizah, M., Somchit, M. N., Teh, L. K., și colab. (2011). In vivo antiulcer activity of the aqueous extract of Bauhinia purpurea leaf. Journal of Ethnopharmacology, 137, 1047–1054.

Zimmer, A. R., Leonardi, B., Miron, D., Schapoval, E., Oliveira, J. R., Gosmann, G. (2012). Antioxidant and anti-inflammatory properties of Capsicumbaccatum: fromtraditional use to scientific approach. Journal of Ethnopharmacology, 139, 228–233.