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Food Health and Technological Sciences is an international open access, peer-reviewed scientific research journal that provides rapid publication of articles in all disciplines of Food Science, Food Technology, Nutritional Science, Food Health Sciences including Cancer Science, Oncology, Public Health, other applied sciences such as Biomedical Engineering, Industrial Engineering, Mechanical Engineering, Material Science and Nanotechnology.

I am very aware of the responsibilities that the editor's role entails, and I approach my new role with so excitement. I would like to point out that the policy of top priority of **FHTS** is especially to put forward and to reveal the innovations and inspiring outputs for food health and technological sciences. **FHTS** offers an exceptionally fast publication schedule including prompt peer-review by the experts in the field and immediate publication upon acceptance.

Not only my deputy editorial concept but also the all editorial board aims the fast reviewing and evaluation of the submitted articles for the forthcoming issues. Our journal distinction is to make difference in this inspection point. **Food Health and Technological Sciences** journal will continue to publish high quality researches on basic sciences and applied sciences.. Original research articles form the bulk of the content, with systematic reviews an important sub-section. We will encourage all authors to work to these standards. Such emphasis on methodological rigour is vital to ensure that conclusions reached from publications contained in the journal are valid and reliable. Peer review processing remains a vital component of our assessment of submitted articles to **FHTS**.

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Lastly I should thank all our submitting authors, who have toiled in the production of their work, and have chosen **Food Health and Technological Sciences** as the journal they would like to publish in. Have a great Publishing with **FHTS** ...

Ozlem Tokusoglu
Journal Editor

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Innovative Food Processing on Food Chemistry, Food Bioactive Composition and Public Health Nutrition

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Abstract

In this review context, innovative food processing including high pressure processing (HHP) and pulsed electrical fields (PEF) effects on food chemical properties and bioactives has been dealt. Innovative food processing technologies can influence the quality and quantity of food quality. Innovative non-thermal technologies (e.g. high-hydrostatic pressure-HHP and pulsed electrical fields (PEF) can preserve the treated foods without decomposing the chemical constituents and sensorial properties which are normally affected during heat treatment and these innovative products are stabil and safety for public nutrition.

Introduction

Consumers around the world are better educated and more demanding in their identification and purchase of quality health-promoting foods. The food industry and regulatory agencies are searching for innovative technologies to provide safe and stable foods for their clientele. Thermal pasteurization and commercial sterilization of foods provide safe and nutritious foods that, unfortunately, are often heated beyond a safety factor that

results in unacceptable quality and nutrient retention.

Nonthermal processing technologies offer unprecedented opportunities and challenges for the food industry to market safe, high-quality health-promoting foods. The development of nonthermal processing technologies for food processing is providing an excellent balance between safety and minimal processing, between acceptable economic constraints and superior quality, and between unique approaches and traditional processing resources (Zhang et al., 2011).

Nonthermal food processing is often perceived as an alternative to thermal food processing; yet, there are many nonthermal preparatory unit operations as well as food processing and preservation opportunities and challenges that require further investigation by the food industry. Nonthermal technologies are useful not only for inactivation of microorganisms and enzymes, but also to improve yield and development of ingredients and marketable foods with

novel quality and nutritional characteristics (Bermudez-Aguirre and Barbosa-Canovas, 2011). Innovative nonthermal processing is effectively combined with thermal processing to provide improved food safety and quality. Nonthermal processing facilitates the development of innovative food products not previously envisioned. Niche markets for food products and processes will receive greater attention in future years.

Nonthermal technologies successfully decontaminate, pasteurize, and potentially pursue commercial sterilization of selected foods while retaining fresh-like quality and excellent nutrient retention. The quest for technologies to meet consumer expectations with optimum quality-safe processed foods is the most important priority for future food science research.

HHP, ultra-high pressure (UHP), and ultra-high-pressure processing (HPP)

are different names and acronyms for equivalent nonthermal processes employing pressures in the range of 200–1000 MPa with only small increases in processing temperature. The UHPs inactivate microbial cells by disrupting membrane systems, retaining the biological activity of quality, sensory, and nutrient cell constituents, thus extending the shelf lives of foods. High pressures inactivate enzymes by altering the secondary and tertiary structures of proteins, changing functional integrity, biological activity, and susceptibility to proteolysis.

HHP processing of dairy proteins reduces the size of casein micelles, denatures whey proteins, increases calcium solubility, and induces color changes (Morris et al., 2007). The use of HHP to increase the yield of cheese curd from milk and accelerate the proteolytic ripening of Cheddar cheeses are promising improvements to the economics for the dairy food industry. The most widely available commercial applications of HHP include pasteurization of guacamole, tomato salsas, oysters, deli-sliced meats, and yogurts. The provision of HHP processing to provide a preservation method for thermally labile tropical fruits is very promising. It is stated that HHP provides pathogen inactivation, shelf-life extension, unwanted enzyme inactivation, gives innovative fresh products, reduced sodium products and clean-labelling.

PEF processing exposes fluid foods to microsecond bursts of high-intensity electric fields, 10–100 kV/cm, inactivating selected microorganisms by electroporation, a disruption of cell membranes. PEF processing reliably results in five-log reduction in selected pathogenic microorganisms, resulting in minimal detrimental alterations in physical and sensory properties of the fluid foods. PEF adequately pasteurizes acid (pH < 4.5) fruit juices and research is continuing on uniform adequate pasteurization of milk and liquid eggs. The commercial

application of PEF to improve the extraction yield of fruit juices and bioactive components of plant materials is in progress. PEF inactivation of enzymes is inconsistent and nonuniform, resulting in plant products subject to short shelf lives at ambient temperatures. It is expressed that PEF provides pathogen inactivation, shelf-life extension of liquid foods, unwanted enzyme inactivation, improves functionality and texture of foods, gives innovative fresh liquid foods and reduced solid volume (sludge) of wastewater. Although PEF is identified as a nonthermal process, temperature increases during PEF processing result in fluid foods at 35–50°C, requiring cooling prior to packaging. The presence of particulates or bubbles in fluid foods subjected to PEF will result in dielectric breakdown, arcing, and scorching of the food. Homogenization and vacuum degassing are necessary to minimize the hazards associated with PEF processing of fluid foods. Technical issues that must be addressed to commercialize PEF for approval as an adequate food pasteurization technology include: (1) consistent and uniform generation of high-intensity electric fields; (2) identification of critical electric field intensities for uniform microbial inactivation; (3) identification of homogenization and vacuum-degassing techniques to assure the absence of particulates and air cells that promote arcing; and (4) identification of flow rates, temperature control, cooling, and aseptic packaging parameters to obtain processing uniformity and safe handling practices (Morris et al., 2007).

High Pressure Processing (HHP)

Phenolic compounds including flavonoids play some important roles in fruits such as visual appearance, taste, and aroma. In addition to these, phenolic compounds have health-promoting benefits. These bioactive compounds have been found to be important in the quality of plant-derived foods (Thomas-Barberan and Espin, 2001). Anthocyanins are a type

of phenolic compounds classified under flavonoids group of phenolic compounds, which are water-soluble glycosides of anthocyanidins (Tokuşoğlu and Hall, 2011).

The flavonoid (Figure 1) composition in fruits is affected by some intrinsic factors, such as using different genus, species, or cultivars, and extrinsic factors, such as the time of the collection of fruits, location, environmental factors, and storage. In addition to these intrinsic and extrinsic factors, some food-processing technologies can also affect the composition of plant phenolics (Tokuşoğlu, 2001).

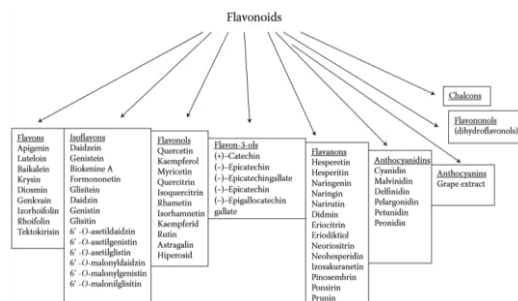


Figure 1. Flavonoid family in food plants. (Adapted from Tokusoglu Ö. 2001. *The Determination of the Major Phenolic Compounds (Flavanols, Flavonols, Tannins and Aroma Properties of Black Teas.* PhD thesis. Department of Food Engineering, Bornova, Izmir, Turkey: Ege University; Tokusoglu Ö., and Hall, C. 2011. *Fruit and Cereal Bioactives: Sources, Chemistry and Applications.* Boca Raton, FL, USA: CRC Press, Taylor & Francis Group, 459pp. ISBN: 9781439806654; ISBN-10:1439806659.)

Phenolic compounds in fruits and vegetables decrease by conventional and traditional heat-treatment processes. These thermal treatments are the most used methods to extend the shelf life of foods by the microorganism and enzyme inactivation, while heat causes irreversible losses of nutritional compounds, undesirable alterations in physicochemical

properties, and changes of their antioxidant properties (Plaza et al., 2006; Wang and Xu, 2007). Many factors including temperature, pH, oxygen, enzymes in the presence of copigments, metallic ions, ascorbic acid (AA), sulfur dioxide, as well as sugars may affect the stability of the anthocyanins. During pasteurization and storage, several red-fruit derivatives lose their bright-red colors and become dull-red colors.

Similarly, the polyphenol content decreases in several liquid, semisolid, or solid foodstuffs by heat treatments (Ferrari et al., 2011). Many food manufacturers have investigated alternative techniques to thermal pasteurization to facilitate the preservation of unstable nutrients and bioactives in foods and beverages. Nonthermal technologies have been reported to be a good option for obtaining food and beverages with a fresh-like appearance while preserving their nutritional quality (Odriozola-Serrano et al., 2009). At that point, the potential use of these emerging technologies, such as “High Hydrostatic Pressure (HHP)” or “Pulsed Electrical Fields (PEF),” are important because they inactivate microorganisms and undesirable enzymes to a certain extent and can avoid the negative effects of heat pasteurization (Toepfl et al., 2006).

HPP can be used to obtain a high-quality food/beverage and increases its shelf life while maintaining its physicochemical, nutritional characteristics, and bioactive profiles (Tokusoglu, 2011; Tokusoglu and Doona, 2011a,b; Tokusoglu et al., 2010).

The technology is especially beneficial for heat-sensitive products (BarbosaCánovas et al., 2005; Tokusoglu and Doona, 2011). HPP can be conducted at ambient or moderate temperatures, thereby eliminating thermally induced cooked off- flavors. Compared to thermal processing, the HPP of foods results in products with a fresher taste, better appearance, and texture. Foods are

processed in batch (for solid products) or continuous and semicontinuous systems (for liquid products) in a pressure range of 50–1000 MPa; process temperature during pressure treatment can be from below 0°C to above 100°C, while exposure time usually ranges from seconds to 20 min (Bevilacqua et al., 2010; Corbo et al., 2009). HPP technology has been successfully applied in several industrial sectors such as meat, seafood, dairy food, fruit juices, fruit, and vegetable products. HPP has been found to inactivate several microorganisms and enzymes. However, it has less effect on lowmolecular-weight food components such as vitamins, pigments, flavoring agents, and other nutritional compounds. HPP conditions in the range of 300–700 MPa at moderate initial temperatures (around ambient) are generally sufficient to inactivate vegetative pathogens for pasteurization processes, some enzymes, or spoilage organisms to extend shelf life. HPP can also increase the extraction capacity of phenolic constituents, and higher levels of bioactive compounds and phytochemicals are preserved in HPP-treated samples (Oms-Oliu et.al., 2012; Tokusoglu and Doona, 2011ab).

The extraction capacity of phenolic constituents has been increased by HHP and HPP-treated samples that retain higher levels of bioactive compounds (Tokusoglu et al., 2010; Tokusoglu and Doona, 2011ab; Zhang et al., 2005).

Studies on HPP effects on total phenolics determined that these compounds were either unaffected or actually increased in concentration and/or extractability, following treatment with HPP.

It has been reported that the anthocyanins of different liquid foods (red-fruit juices) are stable to HHP treatment at moderate temperatures. The nutraceutical and sensorial properties are strictly related to the anthocyanin and polyphenol content in pomegranate juice at room temperature. It was reported that the

stability or preservation of bioactive compounds of red-fruit juices is contradictory. The concentration of red-fruit-based bioactives decreases with the intensity of the treatment in terms of pressure level and processing time (Ferrari et al., 2010).

It was found that HPP treatment at moderate temperatures promoted the extractability of colored pigments and increased the polyphenol levels of fruits (Ferrari et al., 2010).

Although thermal treatments, enzymatic treatments, and other conventional methods have generally been used for eliminating food allergenicity, some treatments result in a degradation of the processing food characteristics, as well as a deterioration in the flavor and taste; for instance, the development of bitterness or an unpleasant odor (Tokuşoğlu and Bozoğlu, 2015). Besides, the enzymatic treatment applications to foods give a high level of protein; this situation is not practicable, especially for meats. High-pressure (HP) processing treatments are novel-processing techniques that have the potential to alleviate the need for thermal processing of foods. High-pressure (400–700 MPa) processing is combined with temperatures around room temperature (5–40°C). It is stated that treatments offer an alternative to high-temperature pasteurization, or chemical preservation and fresh-like properties of foods are preserved. It is known that the current recommended strategy for allergy sufferers is avoidance of allergen foods and also the recommended strategy for manufacturers is the necessity of labeling regarding potential changes in food manufacturing and/or information of ingredient/additive used in food preparation (Tokuşoğlu and Bozoğlu, 2015).

Pulsed electric field processing (PEF) applies short bursts of high-voltage electricity for microbial inactivation and causes no or minimum effect on food-quality attributes. Briefly, the foods being treated by PEF are placed between two

electrodes, usually at room temperature. The applied high voltage results in an electric field that causes microbial inactivation. The applied high voltage is usually in the order of 20–80 kV for microseconds. The common types of electrical field waveform applied include exponentially decaying and square wave (Amiali and Ngadi, 2012; Barbosa-Cánovas et al., 1999). The principles of PEF processing have been explained by several theories including the transmembrane potential theory, electromechanical compression theory, and the osmotic imbalance theory. One of the most accepted theories is associated with the electroporation of cell membranes. It is generally believed that electric fields induce structural and functional changes in the membranes of microbial cells based on generation of pores in the cell membrane, consequently leading to microbial destruction and inactivation. Compared with thermal processing, PEF processing has many advantages. It can preserve the original sensory and nutritional characteristics of foods due to the relatively short processing time and low processing temperatures. Energy savings for PEF processing are also important compared with conventional thermal processing. Moreover, it is environmentally friendly with no waste generated (Amiali and Ngadi, 2012).

PEFs can cause electroporation of cell membranes that, depending on the field intensity, may induce irreversible cell damage. It is stated that PEF can be applied as an alternative method for cell disintegration. Biological tissues exposed to high electric field pulses develop pores in the cell membrane and these actions result in increased membrane permeability and loss of the cell content (Knorr et al., 2001; Tokuşoğlu et al., 2015). It is stated that the novel nonthermal technology PEF for pasteurization or sterilization can inactivate microorganisms and enzymes with minor increasing in temperature,

providing fresh-like products with improved flavor and color properties as well as highly preserved nutritive value (Aguilar-Rosas et al., 2007).

Specific Study on Citrus Juices By HHP and PEF

It was stated that the greater the electric field strength, higher the temperature, or longer the treatment time, the greater the microbial inactivation (Wouters et al., 2001). It is accepted that the pertinent pathogen in citrus juices is generally regarded as *Salmonella* while critical and relatively PEF-resistant microorganisms in orange juice are lactic acid bacteria and pathogenic *E. coli* (Buckow et al., 2013; Parish, 1998).

Hartyáni et al. (2011) stated the physical-chemical and sensory properties of PEF and HHP-treated citrus juices. In the study described by Hartyáni et al. (2011), the physicochemical quality properties (pH, Brix°, electric conductivity, and color), the aroma content of most consumed citrus juices (100% orange, grapefruit, and tangerine juice) were examined (Hartyáni et al., 2011).

The applied technology was pulsed electric field (PEF) treatment with the parameters of 28 kV/cm with 50 pulses; respectively high hydrostatic pressure (HHP) technology with the parameter of 600 MPa pressure for 10 min treatment time. Table 1 shows the physical-chemical properties and total color difference of fruit juices in the case of control, PEF-treated and HHP-treated samples (Hartyáni et al., 2011). Table 2 shows the organic acid content of the fruit juices in the case of control, PEF-treated and HHP-treated juice. In the study reported by Hartyáni et al. (2011), malic and citric acid content did not decrease significantly after the treatments (Table 2). Respectively, in ascorbic acid content, there was a slight difference, but as an advantage of the treatment the vitamin C content was still quite stable (Hartyáni

et al., 2011). It was established that the electronic nose and tongue were able to differentiate each treatment type from the control samples.

Timmermans et al. (2011) reported that the mild heat pasteurization, HP processing, and PEF processing of freshly squeezed orange juice were comparatively evaluated, examining their impact on microbial load and quality parameters immediately after processing and during 2 months of storage. It was found that microbial counts for treated juices were reduced beyond detectable levels immediately after processing and up to 2 months of refrigerated storage. Quality parameters such as pH, dry-matter content, and Brix were not significantly different when comparing orange juices immediately after treatment and were, for all treatments, constant during storage time (Timmermans et al., 2011). It was stated that the quality parameters related to PME inactivation, such as cloud stability and viscosity, were dependent on the specific treatments that were applied. It was found that mild heat pasteurization was effective and was obtained as the most stable orange juices (Timmermans et al., 2011). On the basis of the data obtained by Timmermans et al. (2011), residual enzyme activity was clearly responsible for changes in viscosity and cloud stability during storage for PEF. Figure 2 shows the overview of the production, handling, and analysis of orange juice samples (Figure 2).

It was found that mild heat-pasteurized orange juice was significantly lighter than HP and untreated orange juice, having less red color and more yellowness. It was reported that PEF-treated samples showed the opposite: significantly darker in color than untreated, HP and heat, with significantly more red and less yellow tints (Timmermans et al., 2011). It was shown that DE values, indicated in Figure 3, are the sum of L^* , a^* , and b^* values, which are more closely associated to consumer

perception than singular L^* , a^* , or b^* values, since consumers do not judge each particular attribute, but the combination of them (Cserhalmi et al., 2006). According to DE data obtained by Timmermans et al. (2011), it was found that all types of orange juice showed a noticeable (DE 0.5–1.5) difference in color compared to its color on day 1 and there were no noticeable color differences between the different treatments (Figure 3) (Timmermans et al., 2011).

In the study described by Timmermans et al. (2011), PEF-treated orange juice gave a slightly lower Brix° after processing. It was reported that the effect of mild heat treatments on the pH of orange juice was determined and no significant differences were found between untreated and different types of treated juices. It was found that there was no variation during storage time, except for the untreated orange juice, in which the pH decreases significantly over the first 9 days (Timmermans et al., 2011).

As it is known, cloud loss is considered as a quality defect in shelf-stable citrus juices derived from the concentrate and it is one of the main reasons for the level of heating in heat pasteurization, where 90–100% of PME is inactivated (Goodner et al., 1999). It was reported the observed sedimentation and cloud loss of untreated, mild heat-pasteurized, high-pressure-pasteurized (HPP), and PEF-processed orange juice bottles during the first 115 days of storage at 4°C (Timmermans et al., 2011). It was stated that cloud stability was measured evaluating the degree and rate of sedimentation, by recording the height of the interface of the sediment and cloud. On the basis of the data obtained by Timmermans et al. (2011), sedimentation of 0% corresponded to a completely stable orange juice, having no cloud loss.

Conclusion

The requirement of fortified bioactive compounds such as polyphenolic

antioxidants and minor component vitamins has been accelerated the development of innovations in the food industry, generating the so-called ‘functional foods’ and ‘nutraceuticals’ Innovative food processing technologies can influenced the quality and quantity of food quality. Innovative non-thermal technologies (e.g. high-hydrostatic pressure-HHP and pulsed electrical fields (PEF) can preserve the treated foods without decomposing the chemical constituents and sensorial properties which are normally affected during heat treatment. Also by using of novel technologies, the bioactive chemical constituents have been obtained from food waste recovery and it can be utilized as food by product based powders for public nutrition.

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Table 1. Physical–Chemical Properties and Total Colour Difference of Fruit Juices in Case of Control, PEF Treated, and HHP Treated Samples

Sample	Treatment	Brix (%)	pH	Conductivity (mS)	Total Color Difference (ΔE)
Orange	Control	10.60 ± 0.01	3.65 ± 0.01	3.33 ± 0.01	Reference
	PEF-treated	10.60 ± 0.05	3.65 ± 0.01	3.33 ± 0.01	4.8 ± 0.05
	HHP-treated	10.50 ± 0.05	3.63 ± 0.01	Not measured	9.3 ± 0.01
Grapefruit	Control	8.90 ± 0.05	2.96 ± 0.01	3.72 ± 0.01	Reference
	PEF-treated	8.90 ± 0.01	2.96 ± 0.01	3.78 ± 0.01	2.8 ± 0.06
	HHP-treated	8.70 ± 0.01	2.92 ± 0.03	Not measured	2.1 ± 0.05
Tangerine	Control	10.20 ± 0.05	2.95 ± 0.01	3.50 ± 0.01	Reference
	PEF-treated	10.20 ± 0.01	3.00 ± 0.01	3.60 ± 0.01	3.9 ± 0.05
	HHP-treated	10.20 ± 0.01	2.90 ± 0.01	Not measured	2.6 ± 0.05

Source: Adapted from Hartyáni P. et al. 2011. Innovative Food Science and Emerging Technologies, 12, 255–260.

Note: Values were mean ± SD of three measurements, n = 4; different letters represent a significant difference within the same column (p < 0.05).

Table 2. Organic Acid Content of the Fruit Juices in Case of Control, PEF Treated, and HHP Treated Samples

Sample	Treatment	Malic Acid (mg/l)	Citric Acid (mg/l)	Ascorbic Acid (mg/l)
Orange	Control	847.50 ± 70.06 ^a	5290.73 ± 207.48 ^a	511.59 ± 2.04 ^a
	PEF-treated	826.24 ± 0.09 ^a	5222.57 ± 63.59 ^a	520.64 ± 12.93 ^a
	HHP-treated	755.77 ± 53.83 ^a	5207.17 ± 254.89 ^a	526.29 ± 17.64 ^a
Grapefruit	Control	537.42 ± 49.00 ^a	9923.92 ± 80.86 ^a	421.18 ± 0.79 ^a
	PEF-treated	494.71 ± 5.09 ^a	9933.22 ± 59.90 ^a	411.38 ± 6.96 ^a
	HHP-treated	452.75 ± 49.87 ^a	9751.39 ± 111.82 ^a	405.42 ± 5.56 ^a
Tangerine	Control	903.08 ± 112.20 ^a	341.41 ± 1.41 ^a	6318.03 ± 175.56 ^a
	PEF-treated	944.02 ± 3.55 ^a	346.45 ± 1.15 ^a	6557.13 ± 7.03 ^a
	HHP-treated	1191.20 ± 105.92 ^b	386.49 ± 12.33 ^b	7596.88 ± 171.62 ^a

Source: Adapted from Hartyáni P. et al. 2011. Innovative Food Science and Emerging Technologies, 12, 255–260.

Note: Values were mean ± SD of three measurements, n = 4; different letters represent a significant difference within the same column (p < 0.05).

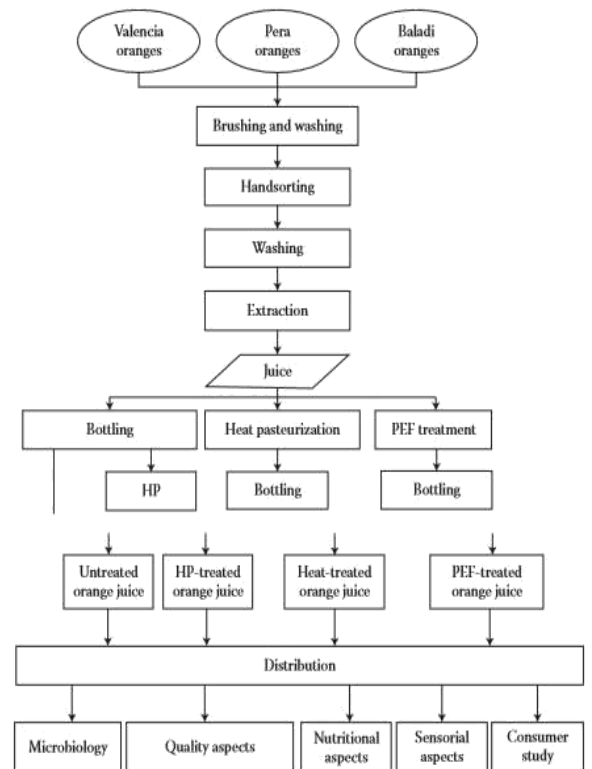
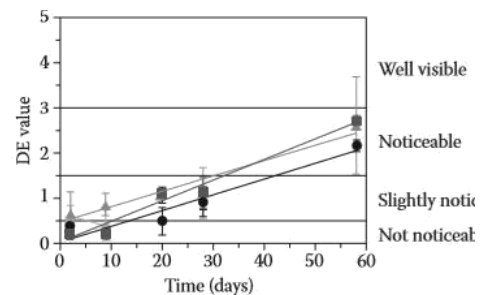


Figure 3. The total color difference (DE value) for untreated (○), mild heat-pasteurized (▲), HP-processed (●), and PEF-processed (■) orange juice. (Adapted from Timmermans R.A.H. et al. 2011. Innovative Food Science and Emerging Technologies, 12, 235–243.)



A New Low Calorie Sweetener D-Tagatose from Lactose in Cheese Whey as a Nutraceutical Value-Added Product

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Abstract

In this manuscript content, a new low calorie sweetener D-tagatose from lactose in cheese whey has been described. These research results are showing progress in improving enzymes bioconversion rate, thermos ability in increasing the half-life of the immobilized enzyme, shorting enzyme reaction time and improving both bioconversion efficiency for the hydrolysis of lactose to lactose hydrolysate (D-glucose and D-galactose) and for isomerization of D-galactose to higher yield of D-tagatose.

1. Introduction

An excess of lactose is currently produced in North America dairy industry as a byproduct of cheese manufacture in the form of whey or whey permeate. These by-products are potential source of food for both human and animal consumption, plus there are technologies for whey utilization that are being investigated. One of these investigated methods is to convert the D-galactose in the disaccharide lactose in whey or whey permeate into the low calorie sweetener D-tagatose via isomerization.

D-tagatose is the keto sugar of D-galactose. It has a sweetness equivalent to

sucrose but it is poorly digested. It is non-cariogenic, reduces insulin demand and can be used as a reduced calorie food sweetening and bulking agent. D-tagatose is also useful as an intermediate chemical for the synthesis of other optically active compounds and as an additive in detergent, cosmetic and pharmaceutical formulations.

The disaccharide lactose in cheese whey can be hydrolyzed by acid or by the enzyme lactase to prepare a mixture comprising of D-galactose and D-glucose at the ratio of 1:1. D-galactose from lactose hydrolysate is the substrate for the production of D-tagatose and it can be separated from D-glucose in lactose hydrolysate by column separation or by relatively converting D glucose in lactose hydrolysate to ethanol by microbial fermentation and distillation.

The separated D-galactose can be isomerized into the low calorie sweetener D-tagatose via chemical method or by enzymatic method. Chemical method is based on using a metal hydroxide such as calcium hydroxide in the presence of an inorganic salt catalyst to form an intermediate metal hydroxide-D-tagatose complex. This intermediate complex is then neutralized with acid to yield D-tagatose (Beadle et.al.,1990). Enzymatic method is based on employing the microbial enzyme L-arabinose isomerase

for isomerization of D-galactose into D-tagatose (Ibrahim and Stradlin,2000).

Currently D-tagatose is generally recognized as safe by North America, Europe, Australian and Asia and these two manufacturing methods of D-tagatose from the substrate D-galactose have been evaluated by worldwide researches for future manufacturing D-tagatose on a large scale.

Researcher's activities are mainly focusing on enzymatic method to improve L-arabinose isomerase properties, activity, and stability in order to improve manufacturing process for cost reduction. Reducing D-tagatose production cost is the key factor for marketing D-tagatose as and an affordable low calorie sweetener for applications in foods, cosmetics and pharmaceuticals industries.

2. D-Tagatose

D-tagatose is a keto hexose, an epimer of D-fructose (Bertelsen et.al.,1999) isomerized at the fourth carbon. The chemical structure of D-tagatose differs from the chemical structure of D-fructose (Bueman et.al.,1998), only in the position of hydroxyl group (OH) on the fourth carbon (Figure 1).

D-tagatose has approximately half the calorie of sucrose (1.5 kcal) and has a sweetness equivalent to sucrose thus it can

be used as a reduced calorie food sweetening and baking agent. Also it is useful as intermediate chemical for the synthesis of other biologically active chemical compounds and it can be used as additive in detergents, cosmetics and pharmaceutical formulations (Levin et.al.,1995).

Functional uses of D-tagatose as food additives are as sweetener, texturizer, stabilizer, humectant, formulation aid, and provides several health benefits to consumers such as attenuate a glycemic response (Armstrong et.al.,2009) and preventing tooth decay.

D-tagatose received a GRAS status (Generally Recognized As Safe) in United States, Australia, New Zealand, South Korea, Brazil, South Africa, the European Union and other parts of the worlds (Lina and Kuper,2002) due to its history of use naturally in foods with no reported adverse effects (Adachi,1958) and for Its naturally existing at a low concentration in human milk, sterilized and powdered cow's milk (Levin et.al.,1995), fermented dairy products such as yogurt (Hirst et.al.,1949) and it is also, present as a component in plants cell structure such as gum exudate of the cacao tree (*Sterculia setigera*) (Elin and Olarsdottir,2001) and one of sugars component in the chemical structure of the oligosaccharide in lichens of the *Rocella* species (Marshall and Kooi,1957)

3. Methods of D-Tagatose

Manufacturing:

The substrate for the production of the keto sugar D-tagatose is the aldehyde sugar D-galactose (Figure 2)

The conversion of D-galactose into D-tagatose is known by the name isomerization and this conversion process can be practice by chemical isomerization using calcium hydroxide at pH12, or by enzymatic isomerization using the enzyme L-arabinose isomerase.

These two processes for manufacturing D-tagatose can be summarized.

3.1. Chemical Isomerization Method

The D-galactose at a concentration of 30% is isomerized to D-tagatose under alkaline conditions at pH 12 for 2-4 hours using calcium hydroxide in the presence of an inorganic salt as catalyst to form an intermediate “calcium hydroxide-D-tagatose complex”. Treatment the precipitated intermediate calcium hydroxide- D-tagatose complex with sulfuric acid forming calcium sulfate and liberates D-tagatose solution. The filtered D-tagatose solution is demineralized in a cation and anion exchanger. The demineralized D-tagatose solution is concentrated and purified by chromatographic fractionation using a

cation exchanger. The purified D -tagatose fraction is crystallized (Ibrahim and Spradlin,2000), separated by centrifugation, washed by spraying distilled water and dried in drum dryer (Ibrahim and Spradlin,2000; Beadle et.al,1992)

9.3.2. Enzymatic Method

Enzymes are the catalysts of biological process that bring the reaction catalyzed to its equilibrium with more specific reaction in short time with less energy than the chemical method. Chemical method is nonspecific and require an extra steps for purification and decolonization to remove generated impurities and color (Ibrahim and Spradlin,2000).

Converting an aldose or aldose derivatives to a ketose or ketose derivatives are well known. For example, the enzymatic conversion of D-glucose to D-fructose, using the enzyme xylose isomerase (EC 5.3.1.5) is widely practiced on a commercial scale for the production of high fructose corn syrup (Nakamatu and Yamanaka,1969). However, the enzymatic method for converting D-galactose to D-tagatose, using the enzyme L-arabinose isomerase (EC 5.3.1.4) (Ibrahim and Spradlin,2000) was not been developed until the last fifteen years as described in

the granted patent for manufacturing of D-tagatose (Ibrahim and Spradlin,2000).

Immobilized free enzyme of L-arabinose isomerase or cell producing intracellular L-arabinose isomerase can be operated in a continuous, semi continuous or batch operation, consistent with the enzyme system employed the isomerization of 20-60 % D-galactose at PH range of 5.0-7.0 in the presence of metallic ion as an activator such as manganese chloride (5mM) and optimum enzyme temperature range of 50-80 °C (Patrick and Lee,1975). The optimum pH and temperature of the enzyme depends on the microbial source of the enzyme L-

arabinose isomerase. L-arabinose isomerase from thermophilic microorganisms produce thermostable enzyme with higher optimum temperature (Sang-Jae et.al.,2005) comparing to L-arabinose isomerase produced from mesophilic microorganisms. Thermostable L-arabinose isomerase has advantages over mesophilic L-arabinose isomerase in shifting the isomerization equilibrium toward the end product (D-tagatose) and also, preventing the potential microbial contamination in the case of continuous immobilization system.

Isomerization of D-galactose into D-tagatose can be completed in 4-8 hours depending on the enzyme activity and properties. Plus, high yield of D-tagatose

in the isomerization process depends on the concentration of D-galactose as a substrate. Isomerization end products are a mixture of D-tagatose and D-galactose. Separation of D-tagatose from D-galactose in the mixture can be performed by chromatographic fractionation using a cation exchanger column separation methods. Separated and purified D-tagatose can be crystallized, centrifuged, washed and dried in drum dryer. The separated D-galactose fraction from D-tagatose / D-galactose mixture can be recycled for enzyme isomerization to D-tagatose.

3.2.1. L-arabinose Isomerase

L-arabinose isomerase (EC 5.3.1.4) is inducible enzyme (Patrick and Lee, 1975) by the pentose sugar L-arabinose and is produced as an intracellular enzyme. The enzyme can be derived from various microorganisms (Sang-Jae et.al.,2005) include but not limited to Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus pentoaceticus , Lactobacillus fermentum (previously known Lactobacillus gayonii), Aerobacter aerogenes, Bacillus amyloliquefaciens, Bacillus subtilis, Candida utilis, Clostridium acetobutylicum Escherichia coli, Erwinia cativosa , Pediococcus spp, Arthrothacter spp.,and Mycobacterium smegmatis. Many of these microorganisms

are Generally Recognized As Safe (GRAS) and are suitable source of L-arabinose isomerase for the enzymatic method to isomerize D-galactose to D-tagatose.

L-arabinose isomerase is mainly catalyzes the isomerization of the pentose - sugar L-arabinose to keto-sugar L-ribulose. This enzyme is also demonstrated its capability to isomerize the hexose sugar D-galactose at high concentration into D-tagatose (Manzo et.al.,2013) (Figure 3). The high concentration of D-galactose as a substrate for the enzyme L-arabinose isomerase is due to the Km value for the substrate D-galactose. (Km value expresses the binding efficiency of the enzyme to its substrate).

3.2.2. Enzyme Immobilization

Isomerization of D-galactose to D-tagatose can only be made economically feasible by immobilizing thermostable L-arabinose isomerase, at high reaction temperature to achieve a higher conversion rate of D-galactose to D-tagatose and yield (Yan et.al.,2014) of D-tagatose.

Immobilized enzymes are defined as enzymes physically confined or localized in a certain region of space with retention of their catalytic activities, which can be used repeatedly and continually. This definition is applicable to the immobilization of free enzymes (extract), or intracellular enzymes

encapsulated in its microbial, plant or mammalian cells. There are various immobilization techniques that are available for L-arabinose isomerase immobilization. The principle of these techniques are illustrated in (Figure 4).

3.2.3. Methods of Enzymes

Immobilization

Cross linking: Several chemicals serve as reagent for intermolecular are cross linking of enzymes. Glutaraldehyde (Kim et.al.,2001) is the most popular cross-linked reagent for both free and cellular enzymes.

Covalent binding: enzyme amino acid residues that are not part of the enzyme active site or substrate binding site can be used for covalent binding with the enzyme support.

Entrapment: Entrapped enzymes are classified into: **Lattice types** (gel matrices), **Microcapsule type** (synthetic polymer), **Liposome type** (phospholipids), **Hollow fiber type** (hollow fiber), and **Membrane type** (ultrafiltration membrane).

Encapsulation: Incorporate, enzymes or microbial cells in a micro metric scale. Microcapsules have pores with diameters between a few micrometers and a few millimeters: to allow the interaction between the enzyme and the substrate.

Adsorption: Binding enzyme to carrier by physical interaction such as hydrogen bonding, hydrophobic interaction or van der waal's forces.

4. Cheese Whey for the Production of D-Tagatose

A total of over 90 billion pounds of whey are generated in United States as a byproduct of cheese manufacturing, comprises about over 84 billion pounds sweet whey and a 6 billion pounds acid whey at the ratio of 9:1 (Charles Ling,2008). The average composition of whey is approximately 0.3 % butterfat, 0.8 % whey protein, 4.9 % lactose and 0.5% minerals (USA Wisconsin Center for Dairy Research). Cumulatively, there are over 4.1 billion pounds of lactose in cheese whey that can be utilized for the production of D-tagatose. Currently, there are about 726 million pounds of lactose per year are recovered in United States from whey.

Whey is the major byproduct from cheese processing industry and it is estimated that for the production of one pound of cheese it will generate a nine pounds of whey. The current lactose crystal/ powder price in United States is in the range of 20 cents per pound. The chemical structure of lactose (β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose) is a

disaccharide aldehyde sugars composed of D-galactose and D-glucose with β 1--4 glycosidic bond (Figure 5). This glycoside bond that bound D-galactose to D-galactose can be hydrolyzed to produce lactose hydrolysate (a mixture of D-glucose and D-galactose at the ratio of (1:1) by acid hydrolysis method using strong acids such as sulfuric acid or by enzyme hydrolysis method Lactase enzyme (E.C.3.2.1.23) is a beta-D galactoside-galactohydrolase enzyme produced by many microorganisms. Lactase enzyme is intracellular enzyme and can be extracted commercially from yeasts such as Kluyveromyces fragilis and Kluyveromyces lactis or from molds, such as Aspergillus niger and Aspergillus oryzae. The commercial enzyme lactase has an optimum temperature of over 48 °C and an optimum pH of 6.5.

The hydrolysis of lactose can be summarized as follow; 20-40 % lactose powder in water is subject for acid or enzymatic hydrolysis. Acid hydrolysis method (Douglas et.al.,2001) can be carried out using 0.1% w/w of sulfuric acid and incubation at 100⁰C for 1-2 hours followed by neutralization to PH 5.5-7.0. . Enzymatic hydrolysis method (Demaimay et.al.,1978) can be carried out using Lactase enzyme by conventional means (free enzyme in batch process) or a continuous (immobilization process), at

optimum temperature 40-60⁰C and optimum PH 4-6 (the enzyme optimum conditions depends on the microbial source and the properties of lactase enzyme being used).

The end product after lactose hydrolysis by either method (acid or enzyme) is lactose hydrolysate which is a mixture of D-galactose and D-glucose at an equal quantities (Figure 6).

For the production of D-tagatose, the substrate D-galactose can be separated from D-glucose by a column separation using a cation exchange resin or by selective fermentation of the D-glucose to ethanol using bacteria or yeast strains (Ibrahim and Spradlin,2000). In the case of D-glucose fermentation to ethanol, microorganisms for ethanol production consumes first the sugar D-glucose for the production of ethanol and the fermentation should be terminated as soon as the sugar D-glucose is completely consumed into ethanol. The ethanol produced from sugar D-glucose can be separated from the remaining D-galactose by distillation and the remaining D-galactose is concentrated to 40-70 % to be used as a substrate for the isomerization process into D-tagatose by chemical or enzymatic method (Figure 7). The D-tagatose production yield varies from 20-30%

depends on the initial concentration of the substrate D-galactose.

5. D-Tagatose Properties

D-tagatose is virtually odorless, white or almost white, non-hygroscopic crystals and it has almost the same sweetness as sucrose with less than half the calories of sucrose (Figure 8). It naturally exists as a monosaccharide keto sugar with unique properties. The standard specification of D-tagatose is illustrated in (Table 1).

D-tagatose provides health benefits to consumers such as having prebiotic function, attenuating glycemic response (Donner et al.,1999), does not promote tooth decay (Levin, 2002; Livesey and Brown,1996) and has poor absorption in GI and low metabolizing energy. These properties of D-tagatose have gained international attention as a low calorie sweetener for diabetics, obese and for weight-conscious populations.

D-tagatose is stable in food products during processing and storage. It is stable at both room and cold temperatures. D-tagatose undergoes Maillard browning reactions with amino acids that give brown color to baked goods (FAO 2004)

6. D-Tagatose Metabolism

Only about 20% of tagatose is absorbed in the small intestine, the rest passes to the large intestine where it is fermented by the beneficial microflora in the colon into short chain fatty acids such as butyric acid which helps in preventing from colon cancer (Johansen and Jensen,1997). These short chain fatty acid are absorbed into blood stream from the Intestinal tract (Bruemann et.al.,1998), as a source of energy. Also, D-tagatose blocks the digestion of sucrose, maltose, and other carbohydrates causing a small or no increase of glucose level in blood stream after meals (Lu et.al.,2008).

The metabolism of D-tagatose, which is a stereoisomer of D-fructose is in the liver as fructose metabolism (Buemann et.al.,2000). It convert into glucose which stored in the form of glycogen or broken down to produce energy. In addition D-tagatose promote the metabolism of Glucose-6- Phosphate which stimulates and maintain the storage of glucose in the form of glycogens (Espinosa and Fogelfeld,2010) stored in the liver.

It is hypothesized that D-tagatose is metabolized in the liver like fructose with similar pathway, but at a lower rate. .The first step in the metabolism of D-tagatose in the liver as illustrated in (Figure 9) is the phosphorylation of tagatose to tagatose -1- phosphate by the enzyme fructokinase.

This enzyme fructokinase is the same enzyme that phosphorylate fructose in fructose metabolism into fructose-1-phosphate.

Similar to fructose -1- phosphate, the tagatose -1-phosphate is an inhibitor of the enzyme glycogen phosphorylase causing glycogen to remain stored in the liver as a mechanism of action for glycemic control. However, the cleavage of tagatose -1-phosphate is slower than fructose -1-phosphate, therefore D-tagatose ingestion may cause a longer lasting reduction in inorganic phosphate (Pi) and adenosine triphosphate (ATP) levels in the liver comparing to D-fructose metabolism (Bar,2000).

Tagatose -1-phosphate in the tagatose pathway is then undergoes hydrolysis by the enzyme tagatose-1 phosphate aldolase to form dihydroxyacetone phosphate (DHAP) and glyceraldehyde. The formed dihydroxyacetone phosphate (DHAP) is isomerized to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase and the formed glyceraldehyde is phosphorylated to glyceraldehyde 3-phosphate by the enzyme glyceraldehyde kinase. The two similar metabolites of glyceraldehyde -3- phosphate from one molecule of D-tagatose are the intermediate in glycolysis pathway that leads to:

- 1 Glycogen synthesis,
- 2 Oxidized to pyruvate pathway.
- 3 Reduced to lactate.
- 4 Acetyl CoA for the anaerobic citric acid cycle in the mitochondria, for energy generation in the form of ATP.

7. D-Tagatose Applications

D-tagatose has a potential multiple applications in foods such as cereals, diet soft drinks, confectionary, hard and soft confectionaries, baked goods, ice cream, chewing gum, ready-to-eat breakfast, etc., as illustrated in (Table 2). In addition to the above foods use. D-tagatose has the potentially for use as a low calorie oral prescription drugs additive to mask unpleasant taste.

8. D-Tagatose Safety

D-tagatose is Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA) (Bar,2000). Plus, the World Health Organization's Joint Expert Committee on Food Additives (JECFA) has left Acceptable Daily Intake (ADI) for tagatose "unspecified," which means that high intake of D-tagatose does not have any expected long-term toxic effects (FDA,2003).

In sensitive individual, D-tagatose when is taken in doses over 10-15 gm / day could cause mild digestion problems such

as nausea, flatulence and diarrhea. In addition, since D-tagatose is metabolized the same way as D-fructose, it is probably not safe to use by individuals with hereditary fructose intolerance (HFI). However accidental intake of small amount of D-tagatose is not likely harmful. In addition, D-tagatose does not likely trigger allergic reaction in individuals with milk allergy (lactose intolerance) and in compare to D-fructose it is slightly increases uric acid levels, but there is no evidence that it could increases the risk of gout (Bar,2004).

9. D-Tagatose and Health Benefits

Consumption of D-tagatose provides the following multiple health benefits along with antioxidant property, the treatment of type 2 diabetes and control obesity:

1. **Antidiabetic potential:** promoting glycogen synthesis and decrease glycogen utilization. Also , attenuate intestinal glucose absorption
2. **Weight loss:** has net zero value
3. **Increase in HDL:** helps rise the good cholesterol (HDL) and may prevent heart attack.
4. **Prebiotic effect:** only 20 % of ingested D-tagatose absorbed into bloodstream while the remaining 80% promote the growth of beneficial bacteria in the colon.

5. **Dental Health:** *non-fermentable and it does not promote tooth decay.*

10. Conclusions

D tagatose is a rare natural monosaccharide which can be manufactured by the chemical or enzymatic isomerization of D-galactose. D-galactose can be obtained by acid or enzymatic hydrolysis of milk disaccharide sugar lactose, into lactose hydrolysate (D-galactose and D-glucose at ratio 1:1), The D-galactose is the substrate for D-tagatose manufacturing and can be separated from D-glucose by column separation or by selectively converting D-glucose to ethanol. Ethanol can be removed via distillation as a valuable byproduct. The separated aldehyde hexose sugar D-galactose can be isomerized to keto hexose sugar D-tagatose by chemical or enzymatic isomerization. . Enzymatic isomerization of D-galactose to D-tagatose is by the enzyme L-arabinose. Isomerase. Enzyme isomerization is the most promising future process for the large scale production of D-tagatose.

Enzymatic methods for the isomerization of aldose to ketose are widely practiced on a commercial scale for converting aldehyde sugar D-glucose from starch to keto sugar D- fructose, however this enzymatic method for converting aldehyde sugar D-galactose from cheese

why to keto sugar D-tagatose have not been developed on a large bench scale until recently (Ibrahim and Spradlin,2000)..

An estimated of over 90 billion pounds of cheese whey are generated in United States as a byproduct of cheese production and relatively small volume of cheese whey are converted into products with various processes and technologies such as condensed whey, dry whey, dried modified whey, whey protein concentrate , whey protein isolate, lactose permeate and crystalized or dried lactose. The rest of unprocessed cheese whey are used for animal feed rand for land spreading.

Crystalized or powder lactose is the best pure feedstock for the two steps processes in D-tagatose manufacturing from whey. These two steps for D-tagatose manufacturing from whey are lactose hydrolysis and D-galactose isomerization. In addition to pure lactose crystal or powder as a feedstock for D-tagatose manufacturing ,the concentrated lactose permeate after the removal of whey protein and other solids can be used as a feedstock in these two steps process for D-tagatose manufacturing . Concentrated lactose permeate consists mainly of 65-85 % lactose and salts. Utilization of lactose permeate is the cheapest feedstock than lactose crystal or powder for D-tagatose production but required an extra step in

the process for salts removal by desalination process (Ibrahim and Spradlin,2000) using reverse osmosis or ultrafiltration technology. Concentrated lactose permeate contain over 7% ashes.

*D-tagatose is listed as a GRAS ingredient in United States., Europe, Australia and Asia, and in the year 2003 the United States Food and Drug Administration (U.S .FDA) has allowed a health claim for D-tagatose and the risk reduction of dental caries. In addition, the European Union (EU) has been formally approved D-tagatose as a “**novel food ingredient**) without any restriction on usages. Plus the joint FAO /WHO Expert Committee on Food Additives (JECFA) concluded that D-tagatose was-not genotoxic, embryo-toxic or teratogenic, and identified a NOEL of 075 gm / kg body weight (45 gm. D-tagatose/ 60 kg body weight) for human intake.*

The D-tagatose safety is also due to its long history of safe use as one of the components in natural foods that demonstrated no indications of any significant adverse effects related to D-tagatose was published in the publically available literature.

Health professional and consumers believe that low calorie sweeteners including D-tagatose are effective for weight maintenance, weight reduction, managements of diabetes, reduction of

risks associated with obesity, and reduction of dental caries.

Reported health benefits associated with of low calorie sweeteners resulted in the increasing worldwide demands for such sweeteners in foods and beverages, and the 2016 global market analysis for low calorie food products and beverages is reaching to over \$ 9.0 billion and the majority of these products containing high intense zero calorie artificial sweeteners such as aspartame, and sucralose, or low calorie sugar alcohols such as sorbitol and xylitol. Up- to- date the natural low calorie sweetener D-tagatose is failed to compete in this market share of low calorie products due to its production cost that estimated to be in the range of \$4-6 per pound and the estimated market price is over \$10 per pound. This high market price of D-tagatose making difficulties for the application of D-tagatose in low calories food products and beverage

There is a potential in reducing the production cost of D-tagatose as a result of the ongoing research in fields of molecular biology, enzyme engineering and bioprocessing. These ongoing research is aimed to improve the two enzymes lactase (B-D- galactosidase) and L-arabinose isomerase expression (Bueman et.al.,1998), activities, specificities (Marta and Jozef,2012) and thermostability (Yan et.al.,2014) for the two steps process for

the production of D-tagatose from lactose in whey.

These research results are showing progress in improving enzymes bioconversion rate, thermos ability in increasing the half-life of the immobilized enzyme, shorting enzyme reaction time and improving both bioconversion efficiency for the hydrolysis of lactose to lactose hydrolysate (D-glucose and D-galactose) and for isomerization of D-galactose to higher yield of D-tagatose. These researches progress will reduce the D-tagatose production cost (Kim et.al.,2001) and might bring hope for the future marketing this natural low calorie sweetener (D-tagatose) with health benefits into the market and potential applications in flow calories products (Marta and Jozef,2012).

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**A new low calorie sweetener
D-tagatose from lactose in
cheese whey as a nutraceutical
value-added product**

FIGURES

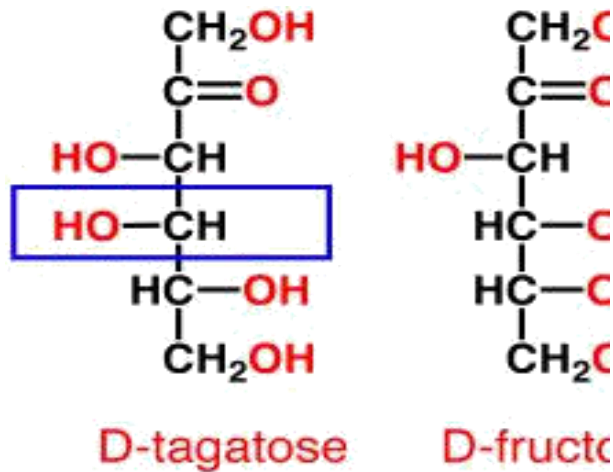


Figure (1): D-tagatose vs, D-fructose

[D-tagatose differs from D-fructose only in the position of hydroxyl group (OH) on 4th carbon]

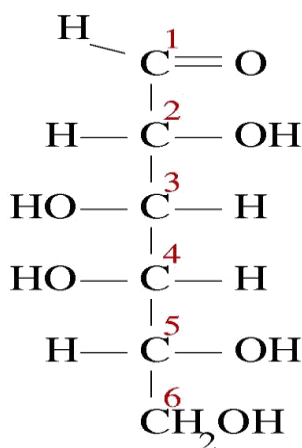


Figure (2): D-galactose chemical structure

[Galactose is a monosaccharide sugar that is less sweet than glucose and fructose. It is a C-4 epimer of glucose]

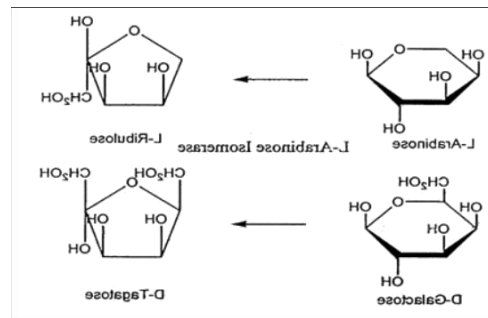


Figure (3): L-arabinose isomerase mechanism of action

[Isomerization of L-arabinose to L-ribose and D-galactose to D-tagatose]

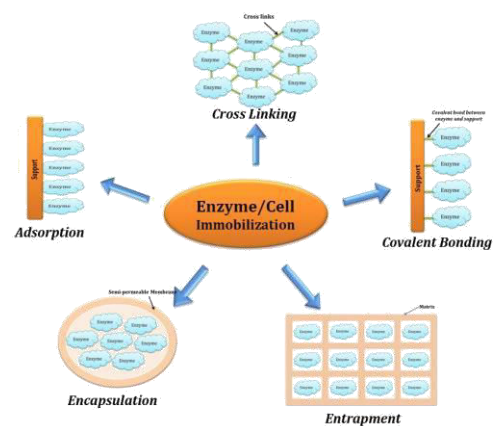


Figure (4): Enzymes Immobilization methods

[Cross linking, covalent binding, entrapment encapsulation and adsorption],

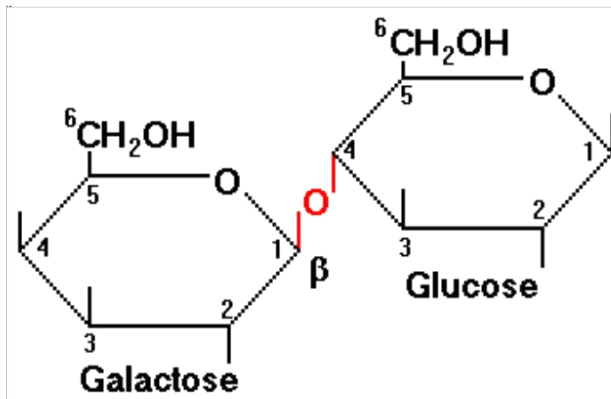


Figure (5): Milk sugar lactose

[Lactose; β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose is a disaccharide sugar composed of galactose and glucose that is found in milk]

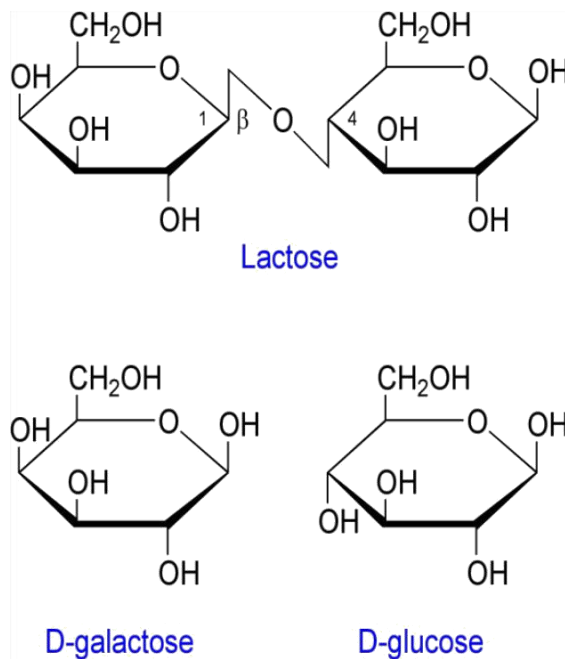
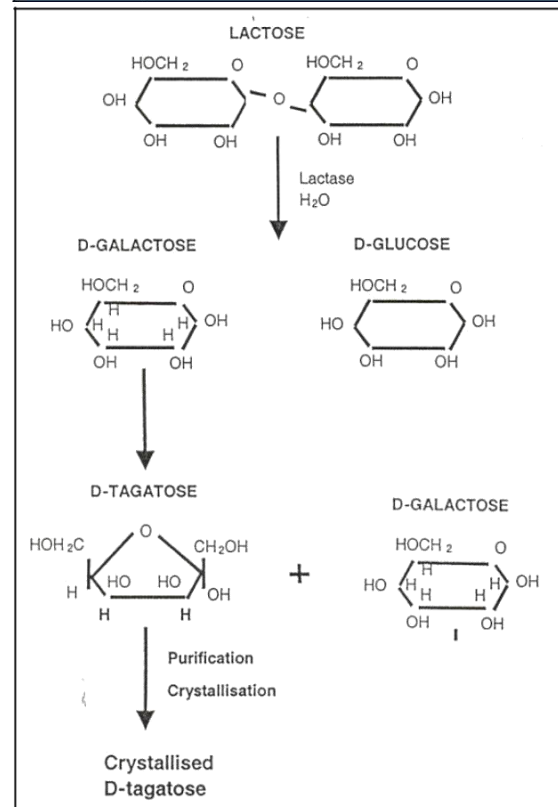
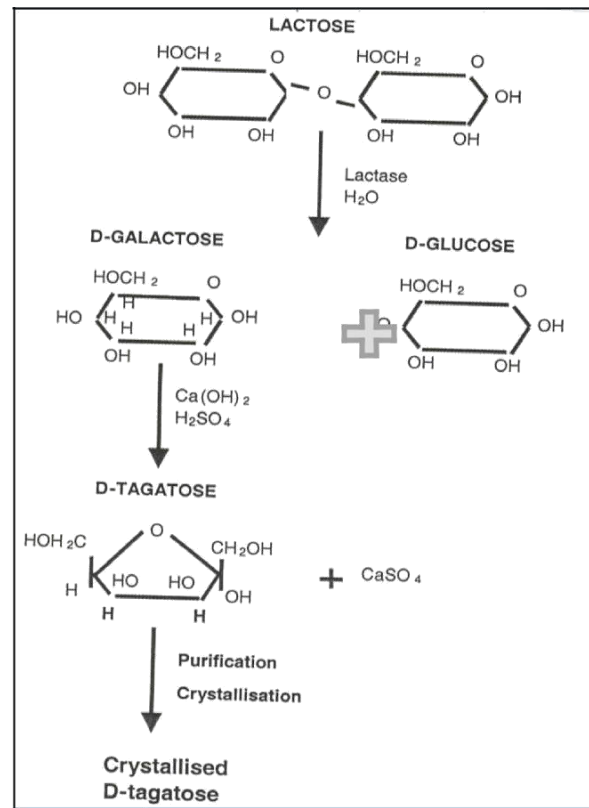


Figure (6): Acid or enzymatic hydrolysis of lactose

[Lactose hydrolysis to D-galactose and D-glucose at ratio 1:1]



Chemical method
Enzymatic method

TABLES

Figure (7): *D-tagatose manufacturing process*

[Diagrams for *D-tagatose manufacturing from lactose in whey by two methods*]

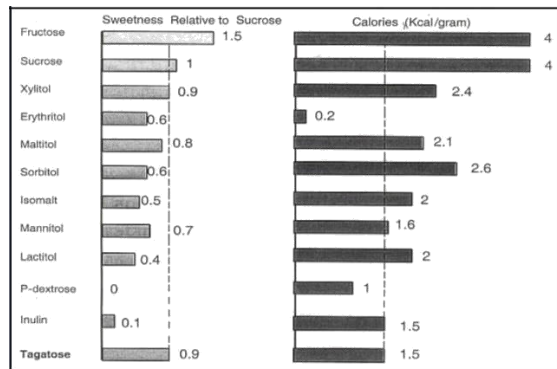


Figure (8): *D-tagatose sweetness and calorie comparing to others sugars*

[*D-tagatose is sweet as sucrose and has 1.5 kcal*]

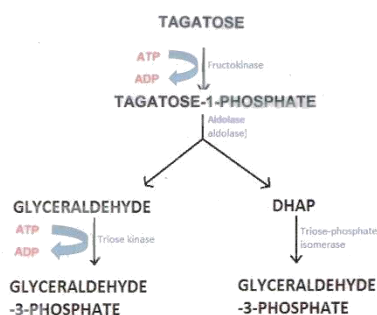


Figure (9): *D-tagatose metabolism*

[*D-tagatose metabolism has a similar pathway as fructose*]

Parameter Specifications

D-tagatose-
98.5% (wt. /wt.)

D-galactose & other sugars
≤ 1% (wt. /wt.)

Moisture
- ≤ 0.5% (wt. /wt.)

Ash
≤ 0.1 % (wt. /wt.)

Solubility in water: at 20
°C 160 mg / 100 ml.

Solubility in ethanol: at 22°C
0.02 gm / 100 ml

Melting point range
133 – 137 °C

Total plate count
<10,000 CFU / g

Coliform
Negative

Staphylococcus aureus
Negative

Heavy metals (Hg, Cd, As)
<0.1 ppm

Lead
< 0.5 ppm

Physical appearance
White Crystal

Table 1: D-tagatose finish product

[Standard specifications]

<u>Food Category</u>	<u>Maximum level of use (%)</u>
Ready-to eat Breakfast cereal	33
Diet soft drinks	2
Non-diet soft drinks	3.
Confectionary	25
Formulated diets for meal replacement	3.
Meal replacement drink mix (powder)	33
Cake, Pie	10.
Cake mixed powder	15.
Frostings	15.
Ice cream and frozen yogurt	7.5
Yogurt	7.5
Chewing gum, sugar free	60.
Jelly and pudding	7.5
Coffee mix powder	7.5

Biscuits
10.

Cookies
10.

Cereal bar
10.

Table 2: The proposed food applications of D-tagatose and maximum level of use

[WHO's Joint Expert Committee on Food Additives (JECFA) has left Acceptable Daily Intake (ADI) for D-tagatose "unspecified," which means that high intake of D-tagatose does not have any expected long-term toxic effects]

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Agri-Food Chain Wastes and Food By-Products: Importance on NutriFood Chemistry and Anticarcinogenity

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Abstract

The diverse types of by-products can be evaluated by various branches of agrifood industry due to their selected desired properties. The pulps, dregs and wastes in food processing depends on the quality of by-product management, while ensuring the environmental protection and sustainability. It has been currently estimated that many cancer-related deaths could be prevented by adequate lifestyle modifications, particularly changes in nutrition and diet. Dietary polyphenols and other bioactive substances have received much attention for their health benefits, including anticancer properties. In this study content, it has been dealt the importance of agri-food chain wastes and food by-products on nutri-food chemistry and anticarcinogenity.

Key words: Agrifood, food, by-product, anticarcinogenity

1.Introduction

Most food waste derivatives from the drink industry (26%), followed by the dairy and ice cream industry (21.3%), the production and preservation of fruits and vegetables (14.8%), the manufacture of grain and starch products (12.9%), the production, processing and preservation of meat products (8%), the manufacture of vegetable and animal oils and fats (3.9%), the production and preservation of fish and fish products (0.4%) (Tokusoglu,2018a).

Food by-products or food industry shelf-stable co-products as liquid, pomace, or powder forms can be obtained from fruits, vegetables, meats, seafoods, milk and dairy, cereals, nuts, fats and oils processing. Those above-mentioned by-products may be evaluated as a source of dietary phytochemicals including phenolic antioxidants, carotenoids, bioactive other polyphenols, dietary fibers, as a source of proteins, peptides and aminoacids, may be evaluated as extruded products. as a sources of collagen, gelatin, and as a sources of various food additive materials (Tokuşoğlu, 2018a). However, the some of by-products can be utilized as compost for plants, can be used as animal feed, can be utilized as industrial materials. By using spray drying methodology or jeotermal drying technology; the vegetables and fruit by products, meat by-products, fish by-products, dairy by-products, cereal and nut by-products can be performed as effectively. For gaining of bioactive components from each processes; powder and hydrolizate flow diagrams have been prepared and applied as firstly and then economical feasibility have been carried out. Then experimental prosedures have been utilized for each bioactive components from food by-products.

2.Fruit and Vegetable By-Products

The current methods for further utilization of product-specific waste of fruits and vegetables have been developed along traditional lines and these utilizations are closely bound to the agricultural origins of the raw materials. Bioactive constituents potentially extractable from the targeted plant food by-products include majorly phytochemicals, fibers, natural flavor compounds, sugars, polysaccharides, ethanol, and proteins and its derivatives. The solid by-product, often called as ‘waste’ or ‘pomace’, is obtained by pressing of fruits or vegetables and can contain pulp, peels, seeds and, stones. The processing of fruits and vegetables results in high levels of waste materials including

peels, seeds, stones, and oilseed meals (Tokuşoğlu, 2018ab).

In the innovative technologies, new aspects regarding the utilizing of above-mentioned wastes as by-products for further exploitation on the manufacturing of high-value products, food additives or supplements with high nutritional value. Due to the high consumption and industrial processing of the edible parts of fruit wastes such as peels and seeds of fruit (apple, pear, orange, pomegranate, tomato) residues, citrus fruit skins as waste, mango residues, pineapple residues, residues of other exotic tropical fruits (avocado, banana, guava, jackfruit, longan fruit), chestnut residues, olive residues, sugarcane bagasse are generated in large quantities in big cities. Besides the peel or leaf or stem fractions of cabbage, cauliflower, celery, Chinese cabbage, coriander, cucumber, eggplant, endive lettuce, fennel, ginger, green pepper, lotusroot, potato, rape, scallion, spinach are utilized as a sources of dietary phytochemicals, dietary fibers by manufactured powder forms and also are used as extruded products (Tokuşoğlu, 2018b).

By-products of fruit and vegetable as a sources of majorly phenolics and dietary fibre and minerals that have a wide range of action which includes antitumoral, antiviral, antibacterial, cardioprotective and antimutagenic activities. Epidemiological studies have pointed out that fruits and vegetable consumption imparts health benefits including certain types of cancer, reduced risk of coronary heart diseases. The health benefits of fruits and vegetables are majorly attributed to bioactive nutrients as phytochemicals, carotenoids, vitamins (ascorbic acid, tocopherol etc.), also to dietary fiber of these products (Tokuşoğlu, 2018a).

Dietary supplements and/or food fortification may be alternative for above-mentioned healthy constituents. By-products of fruits and vegetables are sources of these healthy compounds and it

has been considered these highly desired constituents of by-products of fruits and vegetables.

3. Animal Derived By-Products

The animal-derived wastes include wastes from bred animals, wastes from seafood, and wastes from dairy processing as thirdly. The recovered biomolecules and by-products can be used to produce functional foods or as adjuvants in food processing or in medicinal and pharmaceutical preparations (Tokuşoğlu, 2018ab).

3.1. Meat By-Products

The majority of the by-products in the meat industry is produced during slaughtering. Slaughter house waste consists of the portion of a slaughtered animal that can not be sold as meat or used in meat-products. Such meat by-products includes internal organs, fat or lard, skin, feet, abdominal, the contents of the gastrointestinal tract, blood, bones, tendons and the powders has been produced from these by-products. Appropriate utilization of meat by-products is important for the profitability of the meat sector. Meat by-products are produced by slaughter houses, meat processors, wholesalers and rendering plant (Jayathilakan et.al., 2012; Tokuşoğlu, 2018a).

One of the major by-products of meat is slaughterhouse blood that is an inevitable part of the meat production in food chain and represents a rich source of protein. The physicochemical characteristics and utilization of animal blood in various food and industrial applications has been well explored. The angiotensin-converting enzyme inhibitory, antioxidant, anticarcinogen, antimicrobial, and other bioactive peptides are derived from various slaughterhouse animal blood sources. Furthermore, the effect of enzyme choice, degree of hydrolysis, and peptide sequence or size on the potency of these bioactivity. The by-products of meat containing ash biomass includes

phosphorous (P). It is known that some high phosphorous ash can be in sludge ash, meat and bone meal (MBM) and phosphorous from the biomass ash is very important practical significance for biomass energy, biomass ash disposal and phosphorous resource (Tan and Lagerkvist,2011; Tokuşoğlu,2018ab).

It is reported that by-products including organs, fat or lard, skin, feet, abdominal and intestinal contents, bone and blood of cattle, lambs and pigs represents 66.0, 68.0 and 52.0% of the live weight, respectively. It is determined that many organ meats contain more polyunsaturated fatty acids (PUFAs) than lean tissue while brain, chitterlings, heart, kidney, liver and lungs contain lowest level of monounsaturated fatty acids (MUFA) and the highest level of polyunsaturated fatty acids (PUFA). Meanwhile, chicken visceral wastes are rich sources of PUFA concentrates and, in particular, of omega-3 essential fatty acids.

Bioactive peptides from meat by-products generally contain between 3-20 amino acid residues and various generated peptides are denominated bioactives peptides due to their determined health benefits to the consumers like antihypertensive activity (Tokuşoğlu, 2018a).

3.2. Seafood By-Products

Seafood by-products can be dealt with as fish by-products and shellfish by-products. Fish or shellfish by-products are one of the most important raw materials for food, nutraceutical, pharmaceutical, and biotechnological applications. Seafood product processing discard account for about three-quarters of the total weight of catch. Seafood processing has also been used as a possible waste utilization. It is known that the major components of seafood discard products are tongue, cheeks, stomach, liver of fish, protein bioactives from residual fish, marine bioactive lipid components (omega 3,6, DHA,EPA), fish skin, carotenoid bioactives

and chitinous materials from shellfish products, gut enzymes, flavor products, anti-freeze proteins from seafood blood (Tokuşoğlu, 2018ab).

Fish skin waste but also bones and fins could be used as potential sources to isolate collagen and gelatin. Fish collagen and gelatin are currently utilized in diverse fields containing food, cosmetic, and biomedical industries. Collagen and gelatin are unique proteins compared to fish muscle proteins and they are generally rich (above 80%) in non-polar amino acids including glycine (Gly), alanine (Ala), valine (Val), proline (Pro) aminoacids whereas gelatine generally contains glycine unites, proline and 4-hydroxyproline residues. Collagen and gelatin could be also isolated from bone and fins of fish processing by-products. By-products are represented by fish stomachs and viscera silage and fish sauce. It has been stated that carnivorous fishes have high stomach pepsin contents, and a silage made from minced viscera, or from the separated stomach. By ultrafiltration, concentration, and spray-drying, a cod stomach silage can give a pepsin preparation (Baiano,2014; Tokuşoğlu,2018ab). Fish oil from fish processing waste, and marine fish wastes are rich sources of polyunsaturated fatty acid concentrates and, in particular, of omega-3 essential fatty acids. (Baiano,2014; Tokuşoğlu,2018a).

Shellfish by-products are good sources of antimicrobial compounds and ketocarotenoids; it is known that shellfish derived peptides as bifunctional ingredients (Pezeshk et.al.,2015; Tokuşoğlu,2018a). Shrimp processing leads to massive amounts of shrimp biowaste and the major constituents of the shrimp by-products such as protein, chitin (deacetylated chitosan), lipid, minerals and also valuable carotenoid astaxanthin.

Chitosan, a valuable bioactive compound, has widely used in food, agriculture, biotechnology, cosmetics, medicine and waste treatment. Shrimp

wastewater and especially crab shells are also good sources of astaxanthin and bioactive peptides. Astaxanthin (3,3-dihydroxy- β , β -carotene-4,4-dione) from seafood by-products is a ketocarotenoid oxidized from β -carotene, that plays biological roles and possesses a number of desired properties for food and medical applications owing to it is natural ketocarotenoid, nontoxic, high versatile, hydro and liposolubility property, its attractive pink color, its biological functions as vitamin A precursor and superior antioxidant characteristics (Tokuşoğlu, 2018b).

3.3. Dairy By-Products

Dairy by-product whey is also very good source of peptides with remarkable biological activities. It is stated that main by-products of dairy industry are whey, buttermilk, ghee residue and sometimes skim milk. Whey is an abundant by-product of the dairy industry that corresponds to the liquid fraction remaining after milk clotting and casein removal during cheese manufacturing. Whey includes the lactose and non-casein proteins of milk. It is reported that ovine and caprine whey proteins including β -lactoglobulin (β -Lg) and α -lactalbumin (α -La) and derived-peptides have good biological properties (Tokuşoğlu, 2018ab).

It is reported that whey protein hydrolysates enriched in free amino acids (AAs) and hydrophilic peptides could have been responsible for the raised insulinotropic response of BRIN-BD11 cells. In this context, the potential utilization of whey protein hydrolysates and peptides can be performed as natural complementary approaches; these could be implemented through dietary intervention and food-drug therapies for type 2 diabetes management by inhibiting DPP IV activity and thence increasing the half-life of incretin hormones. It is also stated that the bioactivity of other components of cheese of whey including lactose,

oligosaccharides and minerals is good known (Tokuşoğlu, 2018a).

4. Cereal and Nut By-Products

Cereal (flax seed, barley, oat etc) by-products are also so important and phenolic compound extracted from cereal brans, which antioxidants provide resistance against free radical damage, cancer and cardiovascular diseases. γ -oryzanol from rice bran, which is a potent antioxidant, **a cholesterol reducing agent, a tumor inhibiting agent**, and a preventing agent in menopausal syndrome treatment, β -glucans extracted from barley flour, which improve lipid metabolism, reduce the glycaemic index, and lower plasma cholesterol, lignan concentrates from flaxseed, **which act as anti-cancer, antioxidant**, antibacterial, antiviral, and anti-inflammatory agents (Izydorczyk, and Dexter, 2008; Tokuşoğlu, 2018b).

Recently nut by-products has also very importance in food technology. Especially walnuts are unique due to their perfect balance of n-6 and n-3 polyunsaturated fatty acids (PUFAs), a ratio of 4:1, which has been shown to decrease the incidence of cardiovascular risk. Furthermore, the heart benefits of walnut intake include reducing inflammation and improving arterial function. 9–11. Besides walnut phenolics may also have a protective effect on the susceptibility of LDL cholesterol to oxidative modification and on atherosclerosis. Walnut flour (WF) may be obtained from kernel press-cake. WF provides appreciable amounts of protein. It was shown that glutelins of walnut flour have been shown to be highly digestible. The amino acid (AA) composition of WF is dominated by the acidic AA residues of aspartate and glutamate together with relatively high levels of arginine (Tokuşoğlu, 2018ab).

5. Conclusion

It is concluded that the dried functional powders of food by-products derived from fruits and vegetables, meat,

seafood, milk, dairy products and cereals and as well as their chemical, functional properties, bioactive features and utilizations are great important owing to their possible antioxidative, anticarcinogenic reports. Recent pharmacological evaluations and clinical studies of mentioned derivatives prove their health importance for human nutrition and utilization.

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The Improving Quality and Shelf Life of Table Eggs

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Abstract

In this review manuscript content, the necessity of high pressure processing, microbial stability, preservation and shelf life of table eggs by high pressure has been described. Rheological properties of table eggs by high pressure and high pressure effects on egg phosphovitin, ovalbumin and ovotransferrin has been reported. High pressure effects on foaming properties and on color and texture properties of egg has been executed.

Keywords: Quality, Shelf Life, Table Egg, HHP

Introduction to Table Egg and The Necessity of High Pressure Processing

Whole egg (WE) has excellent nutritional value, especially contains high biological value of protein as compared to any dietary protein sources and egg proteins own all covetable nutritional and functional properties. Liquid egg, homogenized as whole egg or separated into white and yolk, is used as an ingredient and/or as a colorant in a wide variety of processed food products (Tokuşoğlu,2013;ICMSF,1998).

Exclusively, liquid whole egg (LWE) contributes physicochemical characteristics to foods including coagulating, foaming, and emulsifying and gelling (Lee, Heinz & Knorr,1999; Yang & Baldwin, 1995). Due to these important functional properties, LWE can be extensively used as food ingredient and colorant of many foods, such as bakery products, meringues, meat products, chocolate, confectionary products, drinks, infant foods, dressings, noodles and snack food industry. Owing to holding a large quantity of air in the form of fine bubbles, the bubbles of beaten eggs expand in a cake mix and the albumen gives strength to the walls of the air pocket. Egg also contains other nutrients; carbohydrates, vitamins, minerals, phospholipids and other functional lipids. The phospholipids containing yolk confers stability on emulsion of oils and water and utilized in the making mayonnaise, egg phospholipids also used as ingredient of dough and ice cream mix (Tokuşoğlu,2013; ICMSF,1998; Dawson & Martinez-Dawson, 1998; Ahmeda et.al.,2003).

The outer eggshell is made almost entirely of calcium carbonate (CaCO₃) and is covered with as many as 17,000 tiny pores. It is a semipermeable membrane, that allows air and moisture to pass through its pores. Chalaza parts in opposite directions of egg serve to keep the

yolk centered. and it is stated that the more prominent the chalazae, the fresher the egg (Figure 1).

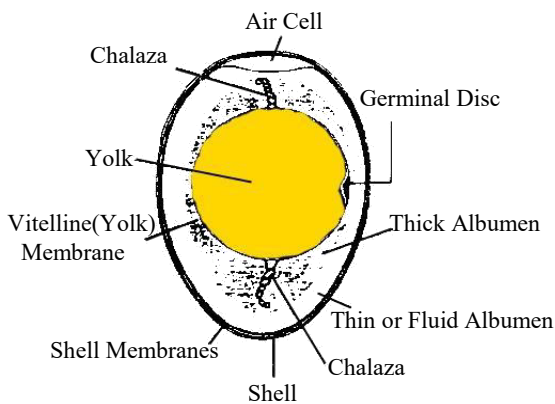


Figure 1. The Parts of Egg

Regrettably, egg and egg products are also responsible for a large number of foodborne illnesses owing to its anatomy and the water, protein and lipid are major components of liquid egg that can support microbial growth at inappropriate storage. Microbial contamination of eggs as well as its economic implications for the poultry industry have been reported (EFSA,2011; Bruce & Drysdal, 1994; Wong & Kitts, 2003).

Traditional thermal treatments used to pasteurize LWE (e.g., 60 °C for 3.5 min in the USA, or 64 °C for 2.5 min in the U.K.) ensure food safety by giving 5-9 Log₁₀ reductions of the most frequent Salmonella serotypes (Alvarez et.al.,2006; Mañas et.al.,2003). Eventhough, some heat-resistant microorganisms can survive the above-mentioned pasteurization requirements and spoil the LWE even under refrigerated conditions (Lee

et.al.,2001). Pasteurisation for LWE is limited to lower pasteurisation temperatures and longer holding times because of the coagulation of its proteins at higher temperatures.

Salmonella is the primary important problem in most cases (EFSA,2011). Recently, Salmonella in eggs has emerged as a primary concern for

public health agencies in Europe and the United States (CDC, 2003, 2004; Schroeder et al.,2005). It was reported that pasteurisation of egg products became mandatory in the US in 1966 (Cunningham, 1995) and current regulations in the US require that LWE is heated to at least 60 C for a minimum of 3-5min. The reason may be attributed to the fact that the USDA requires liquid egg to be heated at the above-mentioned temperature and duration time to achieve more than 3.0 log in colony-forming units (CFU)/mL reduction of Salmonella (ICMSF, 1998). It is concluded that the functional performance of egg white is impaired when heated for several minutes above 57 C (Ma et al.,1997). Owing to the incomplete pasteurization at lower temperatures, the foodborne outbreaks comprising Salmonella enteridis have resulted in eggs (Tood,1996; Tauxe,1991). The illness risk is greater when the egg is used as an food ingredient in foods rather

than when consumed as a individual egg (Todd,2001).

The thermal pasteurization of LWE in most cases leads to protein denaturation and coagulation thence affecting the liquid egg consistency. Therefore, pasteurisation of LWE is limited to lower pasteurisation temperatures and longer holding times owing to the coagulation of its proteins at higher temperatures (Tewari et al., 1999; Tood, 1996; Cheftel, 1995; Tauxe,1991).

High pressure processing (HPP) is an industrially tested technology that offers a more natural, environmentally friendly alternative for pasteurization or shelf life extension of a wide range of food products (Welti-Chanes et al., 2005). The great potential of high pressure processing (HPP) in the food industry has been recently reviewed (Norton and Sun,2008).

Numerously studies including high pressure processing (HHP) technologies have been performed to develop the procedures replacing conventional heat treatment (pasteurization) of liquid egg which is applied at 60-65°C for 5-10 min (San Martín, Barbosa-Cánovas & Swanson,2002; Farr,1990). Using of HHP technology provide the better preservation of native properties of raw foods with similar antimicrobial efficacy as heat treatment. The profitable effects of HHP are demonstrated for many heat sensitive foods and liquid foods are treated in their

packing material to avoid potential postinfection of the final product (Oey et al.,2008 ; Seregély et al.,2007).

Previous studies has shown that high pressure processing (HHP) technology is appropriate for destruction of various pathogen microorganisms in liquid whole egg and egg products (Jankowska et al., 2005; Ponce et al.,1999,1998). The viscosity of egg product is related to coagulation of specific egg proteins induced by HHP, thereby, the pressure is effective on the rheological product characteristics. For effectual treatment of LWE, not only achieving the satisfactory microbiological condition but also preserving the beneficial organoleptic and functional features of LWE are necessary (Tokuşoğlu,2013; Ahmed et al., 2003).

Microbial Stability, Preservation and Shelf Life of Table Eggs By High Pressure

HHP processing inactivates microorganisms, denatures proteins and extends shelf life of food products, with minor effects on nutritional value and flavour. For treatment of LWE, not only the achieving the satisfactory microbiological condition, but also the preservation of its beneficial organoleptic and functional properties are important (Guamis et.al.,2005; Ahmed et.al.,2003).

The HPP exposes the foods with pressures in the range of 100-1000 MPa with processing temperatures from below 0 C to 100 C where significant microbial reduction can be achieved (Huang et.al,2006).

It has shown that pressure treatments (300 - 450 MPa) at various temperatures (15, 20, or 50°C) for 5-15 min efficiently inactivated *Salmonella Enteritidis* inoculated in liquid whole egg (Ponce et al., 1999, 1995).

Bari et.al.(2008) investigated the using of high pressure pulse treatment to inactivate *Salmonella Enteritidis* inoculated in liquid egg. In that study given by Bari et.al.(2008), liquid egg was inoculated with *Salmonella Enteritidis* (8.0 log colony-forming units [CFU]/mL) and exposed to hydrostatic pressures (300-400 MPa) and pressure (350 MPa) pulsing at 25 C, 40 C, and 50 C for up to 40 min to determine the maximum allowable pressure that can inactivate the *Salmonella* with minimal injury. Bari et.al.(2008) stated that strains of *Salmonella* SE-2 and SE-3 were the most sensitive strain to 400 MPa (25 C) pressure treatments for 10 min and a 8.0 and 7.0 log₁₀ CFU/mL reduction were obtained for strain SE-2 and SE-3, respectively. Based on these study, strains SE-1 and SE-4 were the least sensitive and

a 5.0 and 4.0 log₁₀ CFU/mL of inhibition were achieved, respectively.

It was shown that the result of HPP treatment of liquid egg inoculated with *Salmonella Enteritidis* is shown in Table 1 (Bari et.al.,2008).

Table 1. Populations of *Salmonella* Strain SE-4 Recovered from Liquid Whole Egg Following High Hydrostatic Pressure Treatment (Adopted from Bari et.al.,2008)

Treat ment	Population (log ₁₀ CFU/mL) ^a	
	Surv ival	Redu ction
Contr ol	9.12 0.1 2	0.00
300 MPa (30 min)	5.06 0.1 1	4.06 0.11
350 MPa (30 min)	4.37 0.1 0	4.75 0.10
400 MPa (10 min)	3.16 0.1 0	5.96 0.10

^aMean SD (n=3).
Populations of *Salmonella* were recovered on tryptose soy agar medium. CFU, colony-forming units

It was reported that the 300 and 350 MPa of pressure treatment for *Salmonella* Enteritidis in liquid eggs gave

the 4.0 and 4.8 log₁₀ CFU/mL of reduction, respectively, whereas 400 MPa of treatment gave the 6.0 log₁₀ CFU/mL of reduction at 25 C for up to 40 min (Bari et al., 2008). The effects of HPP temperatures (25 C, 40 C, 50 C) on inactivation of *Salmonella* Enteritidis in liquid egg was monitored at 350 MPa pressure up to 40 min. It was found the highest inactivation of *Salmonella* in the liquid egg was observed at 50 C which resulted to a 6.0 log₁₀ CFU/mL reduction (Figure 2).

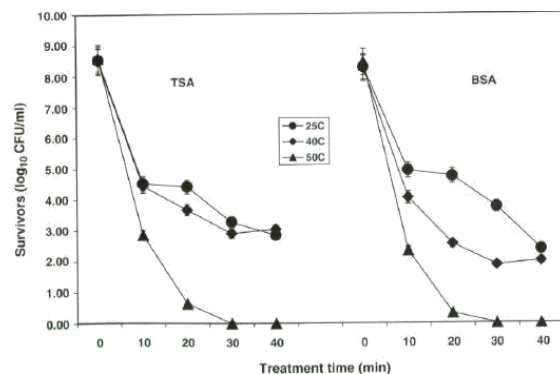


Figure 2. The effects of HPP temperatures on inactivation of *Salmonella* Enteritidis in LWE at 350 MPa/40 min. Values are means \pm SD of three experiments with duplicated determinations (Adopted from Bari et al., 2008)

It was concluded that when the treated liquid eggs were stored at 4 C, 25 C, and 37 C for 24 hours, no *Salmonella* was detected in the samples (Bari et al., 2008)

HPP treatments have been applied to inactivate different microorganisms inoculated in liquid whole egg (Guamis et

al., 2005). It was shown that treatments at pressure above 400 MPa combined with temperature of 50°C were able to reduce *Salmonella* enteritidis count by 8 log₁₀ units whereas total bacterial count was also significantly reduced, and 10 cfu/ml of reduction was detected after 15 days of storage at 4 °C (Guamis et al., 2005).

It was applied 300–450 MPa/ 5–15 min at temperatures of -15, 2, and 20 C to liquid whole egg inoculated with *Listeria innocua* at a pH of 8.0 by Ponce et al. (1998b). *Listeria innocua* inactivation at 400 MPa followed the first-order kinetics for 0–20 min, and exhibited decimal reduction times *D* of 7.35 min at 2 C while 8.23 min at 20 C. The greatest inactivation (5 log reductions) was obtained at 450 MPa for 15 min at 20 C (Ponce et al., 1998b).

Ponce et al. (1998b) stated that the highest reduction of *E. coli* in LWE was obtained at 50 C and it was reported that *E. coli* in LWE was more resistant to pressure at 20 C and -15 C than at 50 C and 2 C (Ponce et al., 1998b).

10⁷- 10⁸ cfu/ml inoculation of *Salmonella* Enteritidis in LWE were subjected to 350 MPa and 450 MPa at 50, 20, 2 and -15 C, with 5, 10, 15 min of treatment times as well as cycles of 5-5 and 5-5-5 min treatments (Ponce et al., 1999). It was concluded that inactivation

increased with pressure and exposure time; the greatest inactivation (8 log cycles) occurred at the severest treatment conditions at 450 MPa/ 50 °C whereas the minimal inactivation (1 log reduction) occurred at the lowest temperature and time conditions (Ponce et.al.,1999).

Lee et.al.(2003) performed the effects of various pressures on *Listeria seeligeri* and *E.coli* (10^7 and 10^8 cfu/ml, respectively), in *LWE* at 5 °C; *Listeria* reductions were not detected after 250 MPa and 350 MPa of treatments for 886 s and 200 s of exposure duration, respectively whereas 2 log reductions of *E.coli* were accomplished (Lee et.al.,2003). Yuste et.al.(2003) reported that 400 MPa/ 5 min of treatments resulted *E.coli* inactivation of 5.5 log cycles in *LWE*, whereas no *Salmonella typhimurium*, *Yersinia enterocolitica*, *Listeria monocytogenes* were detected (Yuste et.al.,2003). Isiker et.al.(2003) stated that increasing the pressure had a significant effect on *Salmonella enteridis* inactivation in *LWE* (Isiker et.al.,2003).

Dong-Un Lee (2002) stated that kinetic studies on the isothermal HHP inactivation of *E. coli* in liquid whole egg were performed at 5 and 25 °C in the pressure range of 250 to 400 MPa. and the characteristic tailing inactivation curves

were described by a first order biphasic model.

It was prevailed that the degree of *E. coli* inactivation at isothermal pressure condition was independent of applied pressures if the physical characteristics of *LWE* are considered, i.e., between 2.0 to 3.0 log reductions at 5 °C, and less than 1.0 log reductions at 25 °C in the range of 250 to 400 MPa, so, HHP at 5 °C is more favorable than at 25 °C (Dong-Un Lee, 2002). It was reported that about 3 log reductions of *E. coli* and over 5 log reductions of *Pseudomonas* and *Paenibacillus*, HHP treatment of *LWE* at 5 °C is regarded to be as effective as conventional thermal pasteurization (Dong-Un Lee, 2002).

Dong-Un Lee (2002) stated that the HHP processing conditions were fixed to either 250 MPa for 886 s at 5 °C or 300 MPa for 200s at 5 °C which have been indicated as the optimized HP processing conditions. It was put forwarded that the addition of nisin (Figure 3) prior to pressure treatments significantly increased the lethal effects of HHP against *Listeria seeligeri*. The individual effects of each nisin and HHP on the *Listeria* reductions were very small, and the increased *Listeria* reductions, up to 5 log cycles were obtained owing to the synergistic action of bactericidal effect of nisin and high pressure effects (Dong-Un Lee,2002).

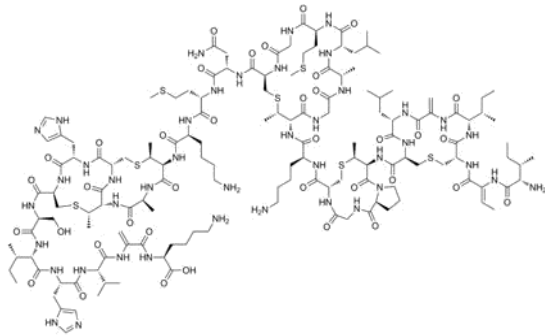


Figure 3. The Chemical Formula of Nisin

The Nisin-HHP combination can be effectively reduced the microbial loads of Gram-negative *E. coli*. It was concluded that the marginal effects of Nisin-HHP synergy on *E. coli* reduction in LWE can be expressed by the membrane structure of Gram-negative *E. coli* or by the protective effects of LWE. (Dong-Un Lee, 2002).

Juliano (2006) studied the inactivation of *Bacillus stearothermophilus* after different stages in the process: (a) baking of egg mix to form patty, (b) after preheating, (c) after high pressure high temperature (HPHT) processing. In the study described by Juliano (2006), *Bacillus stearothermophilus* spore inoculated in the egg mix showed a one log cycle reduction after baking. It was reported that the inactivation of *B. stearothermophilus* (ATCC 7953) spores in egg patties was accelerated after pressure-assisted thermal processing (PATP) treatment at 700 MPa/105°C and *B. stearothermophilus* spores was inactivated, rapidly in egg matrix (4 log reductions in 5 min) when

compared to thermal treatment at 121 °C (1.5 log reduction in 15 min) (Rajan et al., 2006). Similar results was found by Koutchma et al. (2005) and the inactivation of *B. stearothermophilus* in spore strips located between two egg patties can be reduced by at least 6 log cycles at 688 MPa/ 105°C in 5 min (Koutchma et al., 2005).

Rheological Properties of Table Eggs By High Pressure

The egg is a low acid food (higher pH) that necessitates preservation by some means to increase the shelf life. It was found that HHP treated egg white at 600 MPa or more gets fully coagulated to form gels (Bridgeman, 1914).

It is known that the protein can be denatured, coagulated or gelled, and it depends on several factors such as pH, protein type, temperature, applied pressure and ionic strength. HHP has been focused on food proteins, and its functional properties, modification and texture (Ahmed et al., 2003). It is reported that HHP has reduced the alterations of post-process contamination, coagulation and better retention of nutritional qualities of egg as the process is carried out at considerably low temperature and in packed form and also the product could be consumed directly without heat treatment (Ahmed et al., 2003; Knorr, 1996).

Pressure induce protein denaturation, depending on the protein concentration, pressure level, temperature, and pH (Balny & Masson, 1993).

Egg contains protein of high biological value as compared to any dietary protein. Egg proteins have all the desirable nutritional and functional properties, so egg proteins are widely used in food technology (Lee et al., 1999; Hsieh et.al.,1993).

For the food quality control, sensory evaluation, food process and equipment design and also for the new product development; the information of rheological properties of foods is necessary. Based on the origin, chemical and nutritional composition and structure behavior and previous history, the flow behavior of a fluid can be varied from Newtonian to timedependent non-Newtonian in nature (Rao, 1986). It was reported Newtonian and/or time-dependent non-Newtonian flow behavior of egg (Lee, Heinz & Knorr,1999; Comford et.al.,1969) and it was studied the rheology of commercial egg gel white at high temperature using creepand compression measurement (Nagano & Nishinari,2001).

The denaturation or coagulation or structure break down occurs in albumen or egg white protein and the role of protein structure on emulsion and gel rheology is important, so, HHP effects on the

rheological properties of egg is also significant (Ahmed et.al.,2003).

Ahmet et.al.(2003) stated that whole liquid egg (WLE) and albumen have been denatured at high hydrostatic pressure (HHP) and it was reported that 100-400 MPa/30 min of HHP application affected the rheological characteristics of WLE, albumen, and yolk (Ahmet et.al.,2003). In the study given by Ahmed et.al.(2003), an advanced controlled stress rheometer was employed to study the rheological properties at a shear rate of 0– 200 s^{-1} using double concentric cylinder for WLE and albumen while parallel plate geometry was used for yolk with shear rate range of 0–500 s^{-1} . It was stated that both WLE and albumen behaved as time dependent fluids (thixotropic) however HHP reduced time dependency substantially. It was also determined that albumen individually exhibited more pressure effect compared to WLE and thixotropy of yolk significantly varied (p 0:05) during HHP (Ahmet et.al.,2003).

Table 2. *Rate of Thixotropy and Area Under the Curve of Egg Components Obtained from Software (Adapted from Ahmed et.al.,2003)*

<i>Sample</i>	<i>Pressure (MPa)</i>	<i>Rate of Thixotropy (Pas⁻¹)</i>	<i>Area inside the curve (s⁻¹Pa)</i>
<i>WLE</i>	0.101	54.85	556.7
	150	51.12	345.6
	200	49.56	329.6
	250	42.52	144.6
	300	514.9	1618
	350	384.7	1253
<i>Albumen</i>	0.101	287.4	790
	150	34.22	111.7
	200	11.91	63.45
	250	7.87	53.87
	300	81.86	423.4
	350	ND	
<i>Yolk</i>	0.101	21,930	84,840
	150	24,620	91,500
	200	25,768	89,680
	250	ND	
	300	30,460	92,657
	350	36,830	99,096

It was studied the effects of high hydrostatic pressure (HHP) on rheological parameters of whole liquid egg (WLE), albumen and yolk as egg components (Table 3 and 4). It was shown that for egg albumen, the magnitude of yield stress and consistency coefficient decreased during pressurization; however, coagulation of protein reversed the trends. It was determined that all egg samples behaved as thixotropic fluid and the structure break down of egg protein enhances with high pressure and it completed at 300MPa/30 min at 20 °C (Ahmed et.al.,2003).

Table 3. Effect of Pressure on Rheological Parameters of Liquid Whole Egg (LWE) Using Herschel-Bulkley Model (Adapted from Ahmed et.al.,2003)

	Pressure (MPa)	Yield Stress (Pa)	Consistency Coefficient (), Pas ⁿ	Flow Behavior Index (n)	Standard Error
Liquid Whole Egg (LWE) (Shear Rate 0-200 s ⁻¹)	0.101 up	0.536	0.058	0.753	30.11
	0.101 Dn	0.171	0.023	0.959	17.07
	150 up	0.323	0.044	0.876	17.55
	150 Dn	0.165	0.022	0.961	9.48
	200 up	0.400	0.054	0.893	15.55
	200 Dn	0.309	0.019	1.079	13.61
	250 up	0.334	0.324	0.367	19.66
	250 Dn	0.205	0.032	1.094	11.12
	300 up	0.769	0.786	0.276	16.45
	300 Dn	0.832	0.055	0.962	8.63
	350 up	1.676	0.887	0.186	18.44
	350 Dn	0.506	0.049	0.903	7.56

Table 4. Effect of Pressure on Rheological Parameters of Egg Components Albumen and Yolk Using Herschel-Bulkley Model (Adapted from Ahmed et.al.,2003)

Egg Components	Pressure (MPa)	Yield Stress (Pa)	Consistency Coefficient (), Pas ⁿ	Flow Behavior Index (n)	Standard Error
Albumen (Shear Rate 0-200 s ⁻¹)	0.101 up	0.768	0.032	0.87	16.77
	0.101 Dn	0.536	0.015	1.039	13.21
	150 up	0.096	1.43E-3	0.954	2.57
	150 Dn	0.086	1.28E-3	0.977	2.99
	200 up	0.053	4.016E-3	0.914	16.13
	200 Dn	0.051	3.00E-3	0.969	15.88
	250 up	0.033	2.41E-2	0.564	15.22
	250 Dn	0.027	1.83E-3	1.023	11.77
	300 up	0.220	0.642	0.248	27.33
	300 Dn	0.741	0.016	0.851	19.19
Yolk (Shear Rate 0-500 s ⁻¹)	0.101 up	10.68	21.36	0.473	53.45
	0.101 Dn	9.529	7.363	0.514	6.85
	150 up	a			
	150 Dn	10.29	8.13	0.516	5.92
	200 up	a			
	200 Dn	11.26	8.52	0.509	7.068
	250 up	a			
	250 Dn				
	300 up	a			
	300 Dn	21.14	9.38	0.450	6.56
	350 up				
	350 Dn	28.61	10.09	0.448	7.23

High pressure level at 400-600 MPa can cause enough alterations in the viscosity of egg components so that it can become gel with improved quality characteristics than the heat induced gels (Hayashi et.al,1989). It was expressed that pressure induced gels were softer than untreated samples, more elastic without any cooked taste and flavour and there was no destruction of vitamins and formation of lysinoalanine (Hayashi et al.,1989).

High Pressure Effects on Egg Phosvitin, Ovalbumin and Ovotransferrin

Egg yolk phosvitin represents about 7% of the proteins found in egg yolk and is a highly phosphorylated protein of egg (Samaraweera et.al.,2011; Abe et.al.,1982). Phosvitin, a highly phosphorylated glycoprotein and represents the major fraction of hen egg yolk phosphoproteins (Anonymous,2013). It is known that phosvitin is rich in serine residues and phosphorylated peptides, i.e. phosphopeptides,with antioxidant and mineral-binding ability could be a great source of natural functional biopeptides (Jiang & Mine,2000).

Volk et.al.(2012) stated that phosvitin structure maintained overall during high-pressure treatment of 600 MPa applied at an initial temperature of 65 C regardless of the pH and treatment duration, confirming the high structural

stability of the phosphoprotein. It was reported that treatment of phosvitin with phosphatase increased the degree of dephosphorylation from 24% to 63%, after 2 and 18 h, respectively. It was also found that angiotensin-converting enzyme (ACE) inhibition and antioxidant activity of dephosphorylated and protease-treated phosvitin was increased by 52% and 39%, respectively, as compared to protease-digested native phosvitin.

It was showed that pressure treatment of egg white proteins above 450 MPa resulted in a loss of secondary structure (Hayakawa et al., 1996). The pressure-induced structural alterations in egg white proteins can also be demonstrated by exposing of previously buried SH groups and hydrophobic groups (Van der Plancken et al., 2004, 2005ab, 2006 ; Iametti et al., 1999). Iametti et.al.(1999) stated that the treated albumen had increased viscosity but retained its foaming and heat-gelling properties (Iametti et al., 1999). It was found that susceptibility of egg albumen proteins to hydrolysis by trypsin increased dramatically after HHP treatment (up to 10 min at 800 MPa).

As it is known, ovalbumin (OVA) (Figure 7) is the major protein found in egg white, making up 60-65% of the total protein (Huntington & Stein, 2001) and it plays a major role in determining of egg

white behavior on application of HHP (Messens et al., 1997). High pressure can result in structural modification of egg white that can be correlated to enhancement of functional properties (Messens et al., 1997). The S-form of ovalbumin, the presence of which is an index of egg aging, was not found in any of the pressure-treated samples, that also did not display evidence for covalent protein aggregation (Iametti et al., 1999).

It was reported that the foaming capacity of egg white have been improved owing to the exposure of SH groups that favours foaming stability and capacity (Van Der Plancken et al., 2007a; Yang et al., 2010). It was reported that the turbidity, surface hydrophobicity and exposure of sulfhydryl groups in egg white proteins were also increased at pressures over 400 MPa application and the strong increase in surface hydrophobicity was observed between 400 and 700 MPa (Yan et al., 2010 ; Van der Plancken et al., 2005a, 2007b). Besides, the decrease and increase of total and exposed SH groups, respectively, were enhanced by pressure above 500 MPa (Van der Plancken et al., 2005a). It was reported that the pressure treatment at 410 MPa induced proteins in egg yolk dispersions to aggregate and undergo a sol-gel transition (Aguilar et al., 2007), while the treatment at 600 MPa resulted in the modification of

emulsifying properties without effect on the protein solubility of LDL solutions (Speroni et al., 2005).

It is known that ovotransferrin, accounting for 12–13% of egg white proteins, is a glycosylated protein with an isoelectric point of 6.1 (Huopalahti, López-Fandiño, Anton, & Schade, 2007) and Huopalahti et al. (2007) stated that the ovotransferrin shows 50% homology with mammalian transferrin and lactoferrin, but differs from the other transferrin proteins in its isoelectric point and in the glycosylation pattern (Huopalahti et al., 2007). It was also stated that ovotransferrin is also responsible for the ferric ion transfer from the hen oviduct to the developing embryo (Huopalahti et al., 2007) and it was found that ovotransferrin possessed antifungal activity (Valenti et al., 1985), immunomodulatory and antiviral activity (Giansanti et al., 2002, 2005, 2007), antioxidant and anticancer activities (Ibrahim et al., 2007; Ibrahim & Kiyono, 2009). Ovotransferrin is known a rich source of bioactive peptides and recently, there is an great attention regarding the potential of ovotransferrin as functional food and nutraceutical ingredient (Wu & Acero-Lopez, 2011).

Current researches indicated that sonication could affect the exposure of SH groups of ovotransferrin and could release of potent antihypertensive peptides (Lei

et.al.,2011; Majumder & Wu, 2010). After HHP processing, the conformational and physicochemical alterations are important due to affecting the functional properties of food proteins and also protein bioactivities.

Acero-Lopez et.al.(2012) reported the effect of high pressure treatment on ovotransferrin. and it was determined that HHP treatment caused changes in ovotransferrin structure depending on the pH of the sample (Acero-Lopez et.al.2012). It was focused that the determination of high pressure effect on the structure and physicochemical properties of ovotransferrin concentrate after processing in an acid (pH 3) and in a basic (pH 8) environments. It was found that, a decrease in total sulfhydryl groups and an increase in surface hydrophobicity were observed along with a partial aggregation at pH 8 and pressures higher than 200 MPa. It was also stated the ovotransferrin adopted a molten globule state at pH 3, and associated with a significant increase in surface hydrophobicity and reactive sulfhydryl content (Acero-Lopez et.al.,2012).

Figure 8 shows the alterations in total sulfhydryl groups and in reactive sulfhydryl content (Figure 8) (Acero-Lopez et.al.,2012). It was stated that ovotransferrin treated at 200 MPa at pH 8 shows a total SH content of 4 mol SH/g,

that is close to the control; whereas further increasing pressure led to considerable decrease in the total SH content to around 2 mol SH/g and 0.9 mol SH/g at 400 and 700 MPa, respectively (Fig.8A).

In the study described by Acero-Lopez et.al.(2012), the most evidential alteration in reactive SH content was observed at 600 and 700 MPa where it decreased from 1 mol SH/g to about 0.2 mol SH/g (Fig 8B). Van Der Plancken et.al.(2005b) revealed that decreasing of the total SH groups was probably due to rearrangement of cysteine residues and oxidation of SH groups (Van Der Plancken et.al.,2005b).

In the study reported by Acero-Lopez et.al.(2012), it was found the gradual increase in denaturation peak from control up to 400 MPa (Fig. 9). Figure 9 shows differential scanning calorimetry (DSC) thermogram of ovotransferrin samples treated at various pressures between 200-700 MPa at pH 8 (Acero-Lopez et.al.,2012).

High Pressure Effects on Foaming Properties of Egg

Heated liquid eggs coagulate or solidify (as cakes, breads, crackers), whipped egg white produces airier and lighter products (meringues, marshmallow, angel cake), and emulsified egg yolk

phospholipids and lipoproteins produces special products (mayonnaise, salad dressing and sauces) (Davis & Reeves,2002). It is known that food foaming characteristics of egg albumen are quite good. Ferreira et.al.(1995) stated that foaming properties are evaluated by foaming capacity (FC) and foam stability (FS). For the determination of FC and FS the following formulae are used as shown in below (Chang & Chen,2000):

$$\text{FC (\%)} = (\text{FV}/\text{ILV}) \times 100\% ; \text{FS (\%)} = [(\text{ILV} - \text{DV})/\text{ILV}] \times 100\% ; \text{Drainage (ml)} = \text{LVM} - \text{LVS}$$

where: FV – volume of foam; ILV – volume of the initial liquid phase; DV – volume of drainage

LVM – volume of the liquid phase at t = 60 min after foaming was finished

LVS – volume of the liquid phase at t = 30 s after foaming

Lomakina & Miková (2006) reported that various foods are prepared using egg white, most of them being based on the foaming properties of egg white that are owing to the albumen proteins ability to encapsulate and retain air (Lomakina & Miková,2006). Due to the foaming properties of egg white, new methods have been improved the volume and the stability of egg white foam (Lomakina & Miková,2006).

Foaming is affected by water (Baldwin,1986) by temperature, sugar (Stadelman & Cotterill,1994), by egg yolk

(Kim & Setser,1982), by oil differency and quantity (Stadelman & Cotterill,1994; Kim & Setser,1982) and also by stabilisers and surfactants (Kim & Setser,1982).

It was expressed that pasteurisation of egg albumen decreased the foaming ability and resulted in the quality reduction and volume of angel cake, this is occurred by ovotransferrin denaturation on pasteurisation at 53°C. Hatta et.al.(1997) stated that using of metallic ions (Fe, Cu, Al, or other) and salts of phosphoric and citric acids for the increasing of denaturation temperature and the improvement of the foaming properties of egg albumen after pasteurisation (Hatta et.al.,1997).

It was reported that pasteurised egg white required a longer whipping to attain a foam comparable in specific gravity to the foam from unpasteurised egg albumen (Stadelman & Cotterill,1994). Ma et.al.(1994) stated that effects of chemical modifications on the physicochemical and cakebaking properties of egg white and they reported that the overrun and the foam stability of spray-dried egg white increased significantly by gamma irradiation processing (Ma et.al.,1994). It was reported the time for 50% drainage, an index of the foam stability, increased by irradiation with higher dosages indicating improvement in the foam stability whereas decreased in the overrun at 4 kGy but no

alteration in the foam stability for the frozen egg white (Ma et.al.,1994).

It was indicated that the greater increasing of the foaming power observed in the case of ultrasound high pressure combination may be explained by the homogenisation effect of ultrasound (Knorr et.al.,2004). Knorr et.al.(2004) expressed the foaming capacity of liquid egg white by ultrasound processing due to ultrasound dispersed the protein and fat particles in liquid egg white (Knorr et.al.,2004). The Table 5 shows the combined processing effects on the foaming capacity of liquid whole egg (Knorr et.al.,2004).

Table 5. The Combined Processing Effects on the Foaming Capacity of Liquid Whole Egg (Adapted from Knorr et.al.,2004).

Processing	Power	Stability
	Overrun (%)	Stability (%)
Control	479	52
High Pressure (HHP)	490	56
Nisin- HHP	484	55
Ultrasound-HHP	638	50

Hoppe (2010) reported that foaming properties of egg white solutions (10% v/v) were analyzed with varying levels of pressure and pH. It was found that pressure treatment of 10% egg white solutions at pH 9.11 resulted in a homogenous solution with improved foaming capacity over the control (Figure 10) (Hoppe,2010). Figure 10 shows the effect of HPP on foam overrun at pH 9.11

at 0.1 MPa (control), 600 MPa, and 800 MPa (Hoppe,2010). It was shown that increasing pressure resulted in an increase in foam volume and foam overrun increased significantly ($\alpha = 0.05$) at 800 MPa at all time points (Hoppe,2010).

Hoppe (2010) expressed that the foaming properties of egg white solutions were also highly dependent on pH (Hoppe,2010). It was found that the greatest foam overrun was achieved at pH 4.5 whereas foaming ability was significantly decreased at pH 6 (Figure 11). In the study described by Hoppe (2010), it was also reported the increased foam overrun at pH 4.5 could be attributed to major egg white proteins (ovalbumin and ovomucin) important to foaming properties, ovalbumin and ovomucin, which have respective pI of 4.5 and 4.1. (Hoppe,2010).

It was found that foam stability determined the effect of HPP or pH on egg white foaming properties (Hoppe,2010). In the study given by Hoppe (2010), HPP significantly reduced foam stability with the exception of the 800 MPa 0 time point and this data was in contrast to the study described by Van der Plancken et. al.(2007a) and was found the HHP treatment increased overall foam stability (Van der Plancken et. al.,2007a).

Figure 12 shows the effect of HPP (5 min) on 10% egg white solution foam

stability at pH 9.11 and at 0.1 MPa (control), 600 MPa, and 800 MPa (Figure 12) (Hoppe,2010). In the study described by Hoppe (2010), the increased stability of 800 MPa at the 0 time point was attributed to the increased foam volume and incorporation of liquid in the foam. It was found that the liquid drainage was the greatest over the first 5 minutes post-foam (800 MPa) as indicated by the slope and drop in stability as shown in Figure 12 (Hoppe,2011).

High Pressure Effects on Color and Texture Properties of Egg

Singh & Ramaswamy (2010) reported that L ,a ,b values increased with increasing in pressure intensity for whole egg. In egg yolk, L remained mostly stable and a value decreased whereas b values showed great increasing in yellowness. It was also reported that high pressure processing (HPP) induced an increasing in L (lightness) value and a (redness) value of egg white up to 700 MPa while the b value simultaneously decreased indicating decreasing yellowness with increasing treatment intensity (Singh & Ramaswamy,2010).

It was demonstrated that high pressure processing affected the color values of egg white, egg yolk and whole liquid egg respectively and it was found that L* value increased linearly at all

pressure-time combinations indicating increase in brightness of sample with increasing pressure treatment intensity for egg white (Table 6) (Singh (2012).

Table 6. Hunter L (Lightness) Values of the Egg White and Yolk Subjected to Pressure Level and Treatment Time (Adapted from Singh,2012)

L* Value				
Egg White				
Pressure	Time			
	0	5	10	15
600	58 ± 0.707	63.5 ± 3.5	66 ± 4.24	80 ± 1.14
700	81 ± 1.41	86	85.5 ± 0.70	91.5 ± 0.70
800	86 ± 3.53	89 ± 4.24	91.5 ± 2.12	96 ± 1.41
900	88 ± 2.82	91.5 ± 3.5	94 ± 1.41	100.5 ± 0.70
Egg Yolk				
Pressure	Time			
	0	5	10	15
600	57.4 ± 0.84	56.8 ± 0.28	53.8 ± 0.21	61.1 ± 0.14
700	57.05 ± 1.34	55.7 ± 0.49	51.7 ± 0.35	44.8 ± 0.07
800	55.3 ± 0.98	50.6 ± 0.84	50.6 ± 0.56	51.2 ± 0.07
900	57 ± 1.41	53.1 ± 5	55.1 ± 0.21	58.5 ± 0.28

It was found a* values increased with increasing pressure treatment

intensity whereas there was small increase in b^* value for egg white (Singh,2012). Table 7 shows the Hunter a^* and b^* values values of the egg white subjected to 600-900 MPa of high pressure at 1-15 min of treatment time (Table 7) (Singh,2012).

Table 7. Hunter a (redness) and b (yellowness) Values of the Egg White and Yolk Subjected to Pressure Level and Treatment Time (Adapted from Singh,2012).

a Value				
Egg White				
Pressu re	Time			
	0	5	10	15
600	1.2 ± 0.00 7	2.00 5 ± 0.02 1	2.22 ±0.4	2.26 ± 0.01 4
700	2.8 ± 0.07 7	2.91 ± 0.01	3.12 5 ± 0.03 5	3.13 ± 0.01
800	1.94 ± 0.65	2.06 5 ± 0.02 1	2.00 5 ± 0.00 7	2.35 ± 0.07
900	1.9 ± 0.02	2.09 5 ± 0.02 1	20.0 25 ±0.0 07	2.25 ± 0.07
Egg Yolk				
Pressu re	Time			
	0	5	10	15
600	9.51 ± 0.01 4	8.46 ± 0.05	8.40 5 ± 0.00 7	61.5 ± 0.07
700	8.63 ± 0.04	5.84 ± 0.06	5.36 ± 0.05	4.15 ± 0.07
800	4.15 ± 0.07	2.95 ± 0.07	2.6 ± 0.28	1.85 ± 0.07

900	3.95 ± 0.07	2.76 5 ± 0.91	2.36 ± 0.06	2.11 ± 0.01 4
b Value				
Egg White				
Pressu re	Time			
	0	5	10	15
600	1.9	1.61 ± 0.01 4	1.80 5 ± 0.00 07	2.25 ± 0.07
700	1.5 ± 0.14	1.19 ± 0.01 4	1.11 ± 0.01 4	1.25 ± 0.07
800	1.3 ± 0.07	1.11 ± 0.01 4	0.98 ± 0.01 41	0.85 5 ± 0.02 1
900	0.99 ± 0.01	1.08 5 ± 0.02 1	1.61 5 ± 0.02 1	2.21 4 ± 0.00 21
Egg Yolk				
Pressu re	Time			
	0	5	10	15
600	44.1 9 ± 15.2	56.1 5 ± 1.20	58.1 ± 0.14	58.1 ± 0.14
700	61.1 ± 0.14	62.5 ± 0.70	62.2 5 ± 1.06	63.4 ± 1.27
800	64.7 5 ± 0.35	66.5 ± 3.53	70.4 ± 0.28	70.6 ± 0.28
900	69.3 ± 1.83	70.5 ± 2.12	72.5 ± 2.12	75.5 ± 0.70

Singh (2012) reported that egg yolk containing high level of xanthophylls (yellow color) showed different color behavior than that of egg white and egg yolk color changed from pale yellow to

orangish yellow as per visual appearance (Singh,2012). It was found that L^* value remained constant and a^* value decreased from 9.51 to 2.11 indicating diminution in redness of sample whereas b^* value increased significantly ($p < 0.05$) from 56.5 to 76.5 showing great deal of increasing in yellow color of the egg yolk (Table 6,7) (Singh,2012). It is known that yellower color is desirable from a customer viewpoint.

It is known that ΔE is the total color difference, it has been represented the color variance of foods during processing (Equation 1). ΔE is obtained as the combined differences in L^* , a^* , and b^* values and ΔE is calculated using L^* , a^* and b^* values whereas raw egg components acted as reference (Azarpazhooh and Ramaswamy, 2011).

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

(Equation 1).

Ahmed et al. (2005) found that ΔE remained constant even after increasing in high pressure processing treatment and this situation indicated the stability of pigments (Ahmed et.al.,2005). It was concluded that ΔE increased with increasing in pressure level and treatment time (Singh,2012).

It is valued that texture is an imperative characteristic of egg and egg

based products that affects consumer perception and overall acceptability. Textural alterations in egg constituents are very sensitive to food processing types and utilized parameters (Kilcast & Lewis,1990; Hayashi et.al.,2000; Tokuşoğlu,2013). Pons & Fiszman (1996) stated that HPP can be used to modify food proteins in controlled manner so as to make egg gels with better quality, uncooked flavor and better textural properties. HPP not only improved the color but also resulted in a more firmer texture than heat coagulated egg products (Singh & Ramaswamy,2010).

With the increasing in pressure level and treatment time, texture properties including firmness, springiness, cohesiveness, gumminess and chewiness improved for egg white. For egg yolk; firmness, adhesiveness, gumminess, chewiness, resilience were enhanced while cohesiveness decreased with an increasing in pressure level and treatment severity (Singh & Ramaswamy,2010) (Figure 13). Singh (2012) stated that the texture profiles including hardness, adhesiveness, cohesiveness, chewiness, gumminess and springiness of pressure treated egg white, egg yolk and whole liquid egg samples.

It was reported that hardness of whole liquid egg (WLE) increased with increasing in pressure level and treatment time but hardness values were lower than egg yolk and higher than egg white

(Singh,2012). With the high pressure application, the form of egg gels was very adhesive and elastic in the study described by Singh (2012) and these data was accordant with that of reported by Hayashi et al.(1989). Similarly, it was found that high pressure coagulated egg white gels were more adhesive and elastic than thermally treated gels (Hayashi et al.,1989). According to another study, high pressure processed gels have softer structure than that of thermal treatments (Carlez et al.,1995). It is known that adhesiveness is related to surface properties. In the study reported by Singh (2012), adhesiveness decreased linearly with increase in pressure level and treatment time for egg white (EW).

Singh (2012) found that increasing pressure level from 500-900 MPa affected the adhesion properties of egg constituents and WLE followed an increasing trend while adhesiveness value of EW was two fold to that of the WLE. It was also found that EY samples were more adhesive physically than EW and WLE. For adhesiveness, egg components followed $EY > EW > WLE$ pattern, where EY demonstrated maximum hardness (Figure 14). It was interpreted that, highest increase in egg yolk could be the higher amount of fat level in egg yolk matrix, thus increasing adhesiveness (Singh,2012; Singh & Ramaswamy,2010).

It was found that egg white (EW) showed different behavior than those of egg yolk (EY) and whole liquid egg (WLE) because of their high protein content. It was reported that high pressure processing (HPP) caused egg white coagulation and increasing the intensity of pressure level and treatment time caused the egg white gelation (Singh,2012).

It was reported that EW turned to opaque at 600MPa/15 min of HHP treatment and was able to form egg gels that can stand by themselves. EY was able to form gels at 700MPa/15 min while WLE was able to form gels at very short time processing treatment of 700MPa/10 min (Singh,2012).

Singh (2012) reported that egg constituents changed from liquid state to complete gel with coagulation and gelation of egg constituents by increasing pressure application. It was stated that egg gels were formed at high pressure level greater than 600 MPa at temperature well below that required for thermal gel formation (Singh,2012). It was concluded that HHP lead to formation of full set egg gels with improved physicochemical and functional characteristics and without any cooked flavors.

Hoppe (2010) reported that egg gels formed with heat at 95°C had an average hardness value and over twice the value of HP-induce gels at 800 MPa

(Figure 15) while the softest gel was observed at 600 MPa (Hoppe,2010). It was also found that pH reduction decreased the hardness of heat induced gels while it increased the hardness of HHP gels in the study described by Hoppe (2010). Egg gel gumminess was determined and was found with similar pattern to gel hardness with heat induced gels being gummier (Figure 15). Figure 15 shows the effect of heat and HHP treatment on egg white gel hardness and gel gumminess (Hoppe,2010).

Monfort et.al.(2012) reported that design and evaluation of a high hydrostatic pressure combined process for pasteurization of liquid whole egg (LWE). It was put forwarded the physicochemical and functional properties of non-treated LWE and HHP treated LWE (300 MPa/3 min at 20 °C) followed by heat treatment (52 °C/3.5 min or 55 °C/2 min) in the presence of 2%, and current heat ultrapasteurization treated LWE (Table 8) (Monfort et.al.,2012).

Table 8. The Physicochemical and Functional Properties of Non-treated LWE, HHP Treated LWE (300 MPa/3 min at 20 °C) Followed by Heat Treatment (52 °C/3.5 min or 55 °C/2 min) in the Presence of 2%, and Current Heat Ultrapasteurization Treated LWE (Adapted from Monfort et.al.,2012)

		<i>Control</i>	<i>TC- HHP- HT</i>		<i>Ultrapasteurization</i>
			<i>HHP + 52 C/3.5 min + 2% TC</i>	<i>HHP + 52 C/3.5 min + 2% TC</i>	<i>71 C/1.5 min</i>
Physicochemical Properties					
<i>pH</i>		7.64 ± 0.06	99.9 ± 0.1	99.7 ± 0.4	102.1 ± 0.7
<i>L</i>		35.0 ± 0.4	103.8 ± 0.2	108.0 ± 0.2	118.8 ± 1.7
<i>a</i>		11.4 ± 0.3	114.0 ± 0.2	114.3 ± 0.1	66.2 ± 1.2
<i>b</i>		25.2 ± 0.5	93.6 ± 0.1	98.2 ± 0.1	61.6 ± 1.3
<i>Viscosity (mPa s)</i>		12.7 ± 0.1	156.4 ± 12.4	132.3 ± 0.6	239.4 ± 2.9
<i>Soluble Protein</i>		0.741 ± 0.024	88.3 ± 0.1	88.3 ± 0.1	84.7 ± 0.7
Functional Properties					
<i>Foaming</i>	<i>Foaming Capacity (%)</i>	504.0 ± 1.6	126.5 ± 1.3	126.4 ± 0.6	31.6 ± 0.8
	<i>Foaming Stability (min)</i>	4.68 ± 0.37	218.0 ± 28.3	186.2 ± 5.9	16.0 ± 2.5
<i>Emulsifyin g</i>	<i>Emulsifying Capacity(%)</i>	62.1 ± 0.8	97.5 ± 2.1	95.8 ± 2.5	31.2 ± 0.7
	<i>Emulsifying Stability (min)</i>	80.6 ± 7.1	95.6 ± 2.5	89.0 ± 1.7	88.4 ± 24.7
<i>Gelling</i>	<i>WHC (%)</i>	86.6 ± 0.5	97.3 ± 1.4	99.2 ± 1.4	100.3 ± 0.6
	<i>Hardness (g)</i>	1041.0 ± 44.5	123.2 ± 2.5	100.7 ± 3.6	126.8 ± 2.0

In the study reported by Monfort et.al.(2012), gels from ultrapasteurized LWE were harder than those prepared with LWE treated by HHP, as reported by Van der Plancken et.al.(2005) in egg white. It was found that, in the case of gelling properties, gels of treated LWE showed similar values of hardness and water holding capacity (WHC) (Table 14). Marco-Moles et.al.(2011) rendered that higher hardness values could indicate that protein-based conformational structures may be irreversibly impaired by high-temperature processes. The designed treatment at 52 °C/3.5 min by Monfort et.al.(2012) resulted in harder gels than the treatment at 55 °C/2 min or the non-treated LWE.

Overall, HPP improved the functional and physicochemical properties of egg white, egg yolk and liquid whole egg. HPP is an emerging technology with the potential to increase new functional properties to food products.

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