

Elaboration of active films with whey protein isolate and concentrate

^{1,2*}Ribeiro-Santos, R., ²Motta, J. F. G., ^{1,2}Melo, N. R., ²Costa, B. S. and ^{1,2}Gonçalves, S. M.

¹Federal Rural University of Rio de Janeiro, Institute of Technology, Department of Food Technology, Seropédica, RJ, Brazil

²Fluminense Federal University, School of Industrial and Metallurgical Engineering of Volta Redonda, Department of Agribusiness Engineering, Volta Redonda, RJ, Brazil

Article history

Received: 19 October 2017

Received in revised form:

15 January 2017

Accepted: 16 January 2017

Abstract

Whey protein-based films are biodegradable food packaging, can be edible, and have high nutritional value. Active compounds can be incorporated into the matrix of these films to extend the shelf life of the products. These additives may be essential oils (EOs) that are natural aromatic compounds, with antimicrobial and antioxidant properties. This study aimed to evaluate different conditions for the preparation of whey protein isolate and whey protein concentrate based films incorporated with essential oil. Protein-based films were prepared by the casting method with or without addition of cinnamon EO (1%, w/w). Different concentrations of protein (6.5, 10, 13 and 19%, w/w) and glycerol (3, 5 and 8%, w/w) were tested, as well as the dispersion material (glass, plastic and tetrafluoroethylene plates) of the filmogenic solution. The film forming solutions with high protein concentration gelled up on heating. The films containing 6.5% protein and 3% glycerol showed good results when Teflon® plate was used for drying. Edible films incorporated with active agents are suitable alternatives for food safety and nutrition, once they can ensure the safety of the packaged product.

Keywords

Food safety

Food packaging

Essential oil

Edible film

Cinnamon

© All Rights Reserved

Introduction

Concerns about safe and healthy foods, with high quality and long shelf life have driven several studies on active packaging technology. Active packaging systems that carry food additives such as antimicrobials, antioxidants, and flavoring agents are of great interest and have been widely investigated (Tavares *et al.*, 2014). Many studies have been performed to develop packaging materials capable of retaining the active agent in the polymer network and controlling its release in food (Coma, 2008; Ramos *et al.*, 2012; Teixeira *et al.*, 2013).

Several biopolymers may be used for forming these active packaging. Carbohydrates, lipids, and protein based films are edible films and have been studied by several authors, once they come from renewable and biodegradable materials. Among the protein-based films, whey protein films have stood out (Perez-Gago and Krochta, 2000; Ramos *et al.*, 2012; Bonilla *et al.*, 2013; Fernández-Pan *et al.*, 2013; Teixeira *et al.*, 2014).

Whey proteins have high nutritional value and functional properties, such as emulsifying capacity, solubility, and gelation properties (Pelegri and Gasparetto, 2003; Ngarize *et al.*, 2005; Roman and Sgarbieri, 2007; Sinha, 2007), and protein isolates

and concentrates are the most marketed products. The characteristics of whey protein-based films such as transparency, brightness, and flexibility allow their application as food packaging (Kim and Ustunol, 2001; Lee *et al.*, 2008; Osés *et al.*, 2009). In addition, their functional properties, as barrier properties may be enhanced by the addition of additives, including essential oils (EOs) (Bahram *et al.*, 2013).

Cinnamon EO has proven to be a natural preservative and antioxidant, containing among their major constituents the cinnamaldehyde and the eugenol (Ulbricht *et al.*, 2011). EO, as cinnamon, can be added to the polymer matrix, thus contributing to the preservation and maintenance of food quality (Wannes *et al.*, 2010; Bahram *et al.*, 2013; Oliveira *et al.*, 2013; Abdollahzadeh *et al.*, 2014). In addition, the EOs are classified as GRAS (Generally Recognized as Safe) under section 409 of the Food and Drug Administration (FDA, 2015), thus bringing benefits to food products (Keokammerd *et al.*, 2008; Antunes *et al.*, 2012; Bellik, 2014).

Several studies with whey protein based – film incorporated with EO were performed with different formulation. In Table 1 is a summary of the conditions for the preparation of whey protein based film found in the literature. The percentage of the used constituents such as the solvent, polymer,

*Corresponding author.

Email: ribeirorsantos@gmail.com

Table 1. Studies of whey protein based film

Film	Whey Protein (WP)	Plasticizer	Essential oil	Drying plate	References
WPI	5,7 and 9% (w/v)	3.6:1% (w/v) (WP: plasticizer)	N.U.	Glass plate	Gounga, Ying and Wang, 2007
WPI	5 and 10% (w/w)	1:1% (w/w) (WP: plasticizer)	N.U.	Teflon	Min and Krochta, 2007
WPC	10% (w/w)	0.5, 0.6 and 0.7% (w/w) (WP: plasticizer)	N.U.	-	Javanmard, 2008
WPI	10	30% (w/w)	N.U.	Polyethylene	Osés et al., 2009
WPC	6.5% (w/w)	3% (w/w)	N.U.	Plastic	Yoshida and Antunes 2009.
WPI	7, 8, 9 and 10% (w/w)	30-60% (w/w)	N.U.	Petri dishes	Kokoszka et al., 2010
WPI	10% (w/w)	5% (w/w)	Oregano, clove, tea tree, coriander, mastic thyme, laurel, rosemary, and sage (0.5-9%, w/w)	Glass dishes	Fernández-Pan, Royo and Maté, 2010
WPI	10% (w/w)	40, 50 and 60% (w/w)	N.U.	Teflon	Ramos et al., 2013
WPC	8% (w/w)	3:1% (w/w) (WP: plasticizer)	N.U.	Polyethylene	Soazo et al., 2013
WPC	8% (w/v)	8% (w/v)	Cinnamon (0.8 and 1.5%, w/v)	Glass	Bahram et al., 2013
WPC	10% (w/w)	40, 50 and 60% (w/w)	N.U.	Teflon	Ramos et al., 2013
WPI	8% (w/w)	50% (w/w)	Almond and walnut (0.5 and 1.0%, w/w)	Petri dishes	Galus and Kadzinska, 2016
WPI	7% (w/v)	60% (w/v)	N.U.	Polypropylene	Oymaci and Altinkaya, 2016

N.U. = Not used

plasticizer and active agent, as well as the steps and used material, interfere in the active film formation. Therefore, testing methodologies based on previous researches is important to ensure the formation of the film as a first stage for the development of an active packaging. For this reason, this study had as objective to evaluate different conditions for preparation of whey protein isolate and whey protein concentrate-based films incorporated with essential oil, in order to obtain film with improved visual characteristics for further testing as food packaging.

Materials and Methods

Whey protein

Whey protein concentrate (WPC) and whey protein isolate (WPI) were purchased from Protesa-Proteins and Nutritionals (Glanbia Nutritionals, United States of America), with certificate of analysis sent by the supplier, with 82.8 and 90.0% protein and <8.0 and <1.3% fat, respectively.

Essential oil (EO)

Cinnamon essential oil (*Cinnamomum zeylanicum* Blume.) was commercially purchased (Ferquima Indústria e Comércio Ltda, São Paulo), originated from China, and produced by steam distillation of cinnamon leaves, presenting eugenol as main component. It was used 1% (w/w) of cinnamon EO because in preliminary testing (data not shows) was better concentration incorporated into matrix polymeric.

Elaboration of whey protein based films

The protein-based films were obtained by the casting method, according to Yoshida and Antunes (2009); Rossi-Márquez et al. (2009); Fernández-Pan

et al. (2012); Ramos et al. (2013), with modifications. Different concentrations of protein and glycerol (Sigma-Aldrich) were used, being 6.5, 10, 13, and 19% (w/w); and 3, 5, and 8% (w/w), respectively. Whey protein was dispersed in distilled water and homogenized until complete solubilization. The pH of the filmogenic solution was adjusted to 7.0 with 5N and 1N sodium hydroxide. The solution was heated in water bath (Quimis, Q214M, São Paulo) at 80°C and 90°C for WPC and WPI, respectively, for 30 min to protein denaturation. Then it was cooled in an ice bath to room temperature (25°C). After cooling, glycerol and 1% (w/w) of EO (when used) were added, the mixture was homogenized in Ultra Turrax (T10 Basic, USA) at 14000 rpm, and 5 mL were sequentially dispersed in glass, plastic, and tetrafluoroethylene plates (Teflon®) for drying at room temperature (25°C) for 48h.

Characteristics analyzed

The solutions were evaluated for formation of the protein-based film, and the surface materials were evaluated for ease of removal of the film. The emulsion stability, color, and aroma of both the film forming solution and edible films, when formed, were also evaluated

Analysis of the results

The experimental design was completely randomized. Three replicates were performed for each trial. The factors studied were: WPC (6.5-19%) and glycerol (3-8%).

Results and Discussion

The protein concentration, pH, and temperature are important parameters that alter the degree of



Figure 1. Film forming solutions containing protein levels above and equal to 10% after heating

protein denaturation and formation of films (Lorenzen and Schrader, 2006; Zhang *et al.*, 2016). Among the formulations without addition of EO, the best films were formed with 6.5% (w/w) protein (concentrate or isolate) and 3% (w/w) glycerol (Figure 2) as described by Yoshida and Antunes (2009).

All protein films were transparent and flexible, as also observed by Osés *et al.* (2009) and Ramos *et al.* (2013). Noticeable differences were observed in the appearance of all films at both sides. No brightness was observed on the side facing the plate, whereas the other side was bright. Ramos *et al.* (2013) observed also differences in the appearance of whey protein-based films at both side, which may be due to phase separation that may occur in the filmogenic solution during drying.

Whey protein-based films are intermediate moisture barriers due to the presence of lactose. Therefore, the WPC-based films are more hydrophilic than the WPI-based films. For this reason, the incorporation of EO into the polymer matrix can make them more hydrophobic, leading to lower water vapor permeability (Ghanbarzadeh and Oromiehi, 2008; Bahram *et al.*, 2013).

The film forming solutions (FFS) containing protein levels above and equal to 10% gelled up on heating (Figure 1), thus glycerol homogenization and dispersion of the solutions in the plates for water evaporation was not possible. Similar results were obtained by McHugh *et al.* (1994) using 12% protein.

McHugh *et al.* (1994) have reported minimal temperature condition of 75°C for 30 min to form intact whey protein-based films. The heat treatment allows denaturation of β -lactoglobulin present in greater quantities in the WPC and WPI, whose denaturation temperature is approximately 78°C. However, films at 75°C were weaker than those formed using severe heat treatments (McHugh *et al.*, 1994).

Therefore, temperatures of 80 to 90 °C for 30

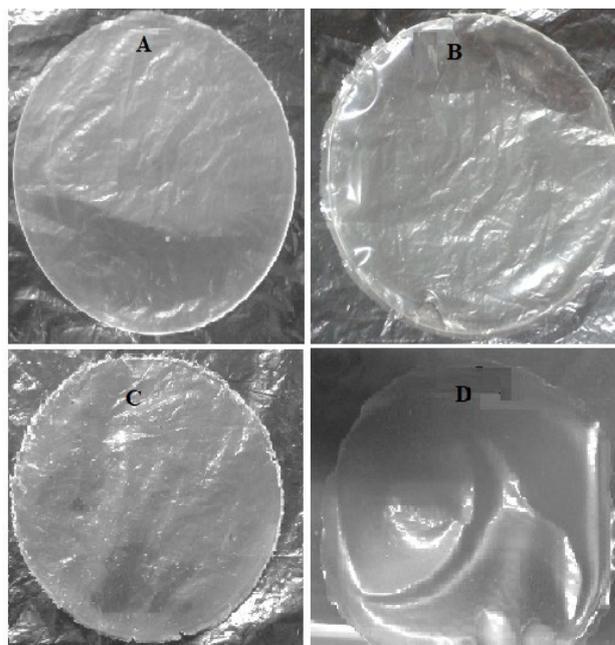


Figure 2. Whey protein concentrated based film without (A) and with (C) cinnamon essential oil (1%, w/w) and Whey protein isolated based film without (B) and with (D) essential oil (1%, w/w).

minutes were used for the formation of WPC and WPI based films, respectively. High temperatures expose the internal hydrogen sulphide group of protein, leading to the formation of intermolecular disulfide bridges (Lorenzen and Schrader, 2006). In the absence of heat treatment, films are cracked into small pieces when dried due to the lack of intermolecular interactions (McHugh *et al.*, 1994).

The pH values in the range of the isoelectric point of whey proteins (pH 4-5) or smaller can lead to insufficient intermolecular bridges for gel formation, probably due to high electrostatic repulsion between proteins, besides increasing water vapor permeability of the films (McHugh *et al.*, 1994; Pérez-Gago *et al.*, 1999; Lorenzen and Schrader, 2006). Pérez-Gago *et al.* (2006) have reported that pH values higher than the isoelectric point cannot affect water vapor permeability. In contrast, McHugh *et al.* (1994) found a significant increase in water vapor permeability at pH 6 when compared to pH 7-9, and FFS at pH 10 can form strong gels, preventing the formation of films.

High water vapor permeability is observed when adjusting pH after protein denaturation, thus the pH of the film forming solution may be adjusted before heat treatment (McHugh *et al.*, 1994). Both FFS and WPI-based films were more white-colored than WPC-based films that were yellowish, probably due to the high fat levels in the WPC when compared to WPI (Ramos *et al.*, 2013), and other impurities including phospholipids and lactose (Banerjee and

Chen, 1995). These impurities can cause changes in some technological properties of the films (Banerjee and Chen, 1995), which can increase permeability and hinder the mechanical properties (Osés *et al.*, 2009).

Unlike what happened to the protein, different concentrations of glycerol used as plasticizer did not interfere in the formation of the films. However, films with high glycerol levels showed a much plasticized texture.

Ramos *et al.* (2013) investigated different glycerol levels and found that WPC and WPI-based films containing 6% glycerol were not easily released from the plate.

The adhesion of the FFS after water evaporation was dependent on the surface material. When dispersed in plastic and Teflon® petri plates, the Teflon® allowed greater ease of removal, possibly because it is a non-stick surface. In contrast, when the solution was dispersed in a glass petri plate, it was unable to detach from the surface. The best films were formed with 6.5% (w/w) protein (concentrate or isolate) and 3% (w/w) glycerol (Figure 2), thus these concentrations were also used for obtaining the films incorporated with EO.

Film incorporated with cinnamon essential oil

Once cinnamon EO has proven to have antimicrobial properties, when incorporated into the polymer matrix, it can extend the shelf life of the product to be packed (Manso *et al.*, 2013; Teixeira *et al.*, 2013), in addition to improving the property of edible films (Peng and Li, 2014). The incorporation of EO (1%, w/w) to the FFS (6.5% protein and 3% glycerol, w/w) intensified the yellow color of both the solutions and films, besides improving aroma due to the volatile compounds present in the EOs.

Both FFS with WPC and WPI containing cinnamon EO were homogenized in Ultra Turrax, allowing an apparent stable emulsion, with smaller and well distributed oil particles, while phase separation was observed when an intensive agitation was not carried out. The FFS containing EO adhered to the plastic petri plate and exhibited brittle appearance. In contrast, the FFS containing EO were easily removed from Teflon® plate, despite the film was dried, brittle, and white-colored. Bahram *et al.* (2013) studied the microstructure of films incorporated with cinnamon EO, and observed many fissures due to evaporation of EO during drying. However, it is possible to obtain protein-based films incorporated with cinnamon EO, as reported by Bahram *et al.* (2013).

Fernández-Pan *et al.* (2012) have reported that the maximum amount that can be incorporated into

the film matrix to form stable films depends on the type of essential oil used. The addition of cinnamon EO led to formation of more yellowish film, with a strong aroma of cinnamon, which tended to increase with increasing the EO levels, thus it is important to determine a maximum OE level to prevent changes in food to be packaged.

Conclusion

Among the concentrations of glycerol and whey protein used for film formation, the best performance was observed for concentrations of 3% and 6.5%, respectively. The use of Teflon® recipient facilitated removal of the film. High protein concentrations in the film forming solutions led to gel formation up on heating. Film incorporated with cinnamon essential oil was formed and it changed the color and flavor of the film. Whey protein-based active films incorporated with essential oil can be edible, besides enriching nutritionally the packaged product. However, studies need to be done in order to verify the effectiveness of the active film and mechanical characteristics from food packaging.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Pró-Reitoria de Pesquisa, Pós -Graduação e Inovação (PROPI / UFF) for the financial support.

References

- Abdollahzadeh, E. Rezaei, M. and Hosseini, H. 2014. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. *Food Control* 35: 177-183.
- Antunes, S. A., Robazza, W. S., Schittler, L. and Gomes, G. A. 2012. Synergistic and antimicrobial properties of commercial turmeric (*Curcuma longa*) essential oil against pathogenic bacteria. *Food Science and Technology* 32(3): 525-530.
- Bahram, S., Rezaei, M., Soltani, M., Kamali, A., Ojagh, S. M. and Abdollahi, M. 2013. Whey protein concentrate edible film activated with cinnamon essential oil. *Journal of Food Processing and Preservation* 38: 1251-1258.
- Bellik, Y. 2014. Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of *Zingiber officinale* Roscoe. *Asian Pacific Journal of Tropical*

- Disease 4(1): 40-44.
- Bonilla, J., Talón, E., Atarés, L., Vargas, M. and Chiralt, A. 2013. Effect of the incorporation of antioxidants on physico-chemical and antioxidant properties of wheat starch-chitosan films. *Journal of Food Engineering* 118: 271-278.
- Coma, V. 2008. Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science* 78: 90-103.
- Food and Drug Administration (FDA) (April 2015). Substances Generally Recognized as Safe (GRAS). Retrieved on November 11, 2015 from FDA Website: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.20.
- Fernández-Pan, I., Royo, M. and Maté, J. I. 2012. Antimicrobial activity of whey protein isolate edible films with essential oils against food spoilers and foodborne pathogens. *Journal of Food Science* 77(7): M383-M390.
- Galus, S. and Kadzinska. 2016. Whey protein edible films modified with almond and walnut oils. *Food Hydrocolloids* 52: 78-86.
- Ghanbarzadeh, B. and Oromiehi, A.R. 2008. Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis. *International Journal of Biological Macromolecules* 43: 209-215.
- Gounga, M. E., Xu, S.-Y. and Wang, Z. 2007. Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formulation. *Journal of Food Engineering* 83: 521-530.
- Javanmard, M. 2008. Effect of whey protein edible film packaging on the quality and moisture uptake of dried peanuts. *Journal of Food Process Engineering* 31: 503-516.
- Keokamnerd, T., Acton, J. C., Han, I. Y. and Dawson, P. L. 2008. Effect of commercial rosemary oleoresin preparations on ground chicken thigh meat quality packaged in a high-oxygen atmosphere. *Poultry Science* 87(1): 170-179.
- Kim, S.-J. and Ustunol, Z. 2001. Sensory attributes of whey protein isolate and candelilla wax emulsion edible films. *Journal of Food Science* 66(6): 909-911.
- Kokoszka, S., Debeaufort, F., Lenart, A. and Voilley, A. 2010. Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films. *International Dairy Journal* 20: 53-60.
- Lee, J.-W., Son, S.-M. and Hong, S.-I. 2008. Characterization of protein-coated polypropylene films as a novel composite structure for active food packaging application. *Journal of Food Engineering* 86: 484-493.
- Lorenzen, P. C. and Schrader, K. A. 2006. Comparative study of the gelation properties of whey protein concentrate and whey protein isolate. *Le Lait* 86 (4): 259-271.
- Manso, S., Cacho-Nerin, F., Becerril, R. and Nerin, C. 2013. Combined analytical and microbiological tools to study the effect on *Aspergillus flavus* of cinnamon essential oil contained in food packaging. *Food Control* 30: 370-378.
- Min, S. and Krochta, J. M. 2007. Ascorbic acid-containing whey protein film coatings for control of oxidation. *Journal of Agricultural and Food Chemistry* 55: 2964-2969.
- McHugh, T.H., Aujard, J.-F. and Krochta, J. M. 1994. Plasticized whey protein edible films: water vapor permeability properties. *Journal of Food Science* 59(2):416-419.
- Ngarize, S., Adams, A. and Howell, N. 2005. A comparative study of heat and high pressure induced gels of whey and egg albumen proteins and their binary mixtures. *Food Hydrocolloids* 19: 984-996.
- Oliveira, M. M. M., Brugnera, D. F. and Piccoli, R. H. 2013. Biofilms in the dairy industries: general aspects and the use of essential oils as a new control alternative. *Journal of the Brazilian Dairy Institute* 68(390): 55-73.
- Osés, J., Fernández-Pan, I., Mendoza, M. and Maté, J. I. 2009. Stability of the mechanical properties of edible films based on whey protein isolate during storage at different relative humidity. *Food Hydrocolloids* 23(1): 125-131.
- Oymaci, P. and Altinkaya, S. A. 2016. Improvement of barrier and mechanical properties of whey protein isolate based food packaging films by incorporation of zein nanoparticles as a novel bionanocomposite. *Food Hydrocolloids* 54: 1-9.
- Pelegrine, D. H. G. and Gasparetto, C. A. 2003. Analysis of whey and egg white proteins solubility. *Brazilian Journal of Agro-industrial Products* 5(1): 57-65.
- Peng, Y. and Li, Y. 2014. Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. *Food Hydrocolloids* 36: 287-293.
- Perez-Gago, M. B. and Krochta, J. M. 2000. Drying temperature effect on water vapor permeability and mechanical properties of whey protein-lipid emulsion films. *Journal of Agricultural and Food Chemistry* 48: 2687-2692.
- Ramos, O. L., Reinas, I., Silva, S. I., Fernandes, J. C., Cerqueira, M. A., Pereira, R.N., Vicente, A. A., Poças, M. F., Pintado, M. E. and Malcata, F. X. 2013. Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. *Food Hydrocolloids* 30: 110-122.
- Ramos, O. L., Silva, S. I., Soares, J. C., Fernandes, J. C., Poças, M. F., Pintado, M. E. and Malcata, F. X. 2012. Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. *Food Research International* 45(1): 351-361.
- Roman, J. A. and Sgarbieri, V. C. 2007. Physical-chemical characterization of whey protein isolate and gelatin from bovine origin. *Brazilian Journal of Food Technology* 10(2): 137-143.
- Rossi-Máquez, G., Han, J. H., García-Almendárez, B., Castaño-Tostado, E. and Regalado-González, C. 2009. Effect of temperature, pH and film thickness on nisin release from antimicrobial whey protein isolate edible

- films. *Journal of the Science of Food and Agriculture* 89(14): 2492-2497.
- Sinha, R., Cheruppanpullil, R., Prakash, J. and Kaultiku, P. 2007. Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. *Food Chemistry* 101(4): 1484–1491.
- Soazo, M., Pérez, L. M., Rubiolo, A. C. and Verdini, R. A. 2013. Effect of freezing on physical properties of whey protein emulsion films. *Food Hydrocolloid* 31: 256-263.
- Tavares, F. O., Pieretti, G. G., Antigo, J. L., Pozza, M. S. S., Scapim, M. R. S. and Madrona, G. S. 2014. Edible coating with oregano and rosemary essential oils for use in ricotta cheese. *Journal of Candido Tostes Dairy Institute* 69(4): 249-257.
- Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M. F., Saraiva, J. A. and Nunes, M. L. 2013. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Industrial Crops and Products* 43: 587-595.
- Ulbricht, C., Seamon, E., Windsor, R. C., Armbruester, N., Bryan, J. K., Costa, D., Giese, N., Gruenwald, J., Iovin, R., Isaac, R., Serrano, J. M.G., Tanguay-Colucci, S., Weissner, W., Yoon, H. and Zhang, J. 2011. An evidence-based systematic review of cinnamon (*Cinnamomum* spp.) by the natural standard collaboration. *Journal of Dietary Supplements* 8(4):378-454.
- Wannes, W. A., Mhamdi, B., Sriti, J., Jemia, M. B., Ouchikh, O., Hamdaoui, G., Kchouk, M. E. and Marzouk, B. 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flow. *Food and Chemical Toxicology* 48(5): 1362-1370.
- Yoshida, C.M.P. and Antunes, A. J. 2009. Application of whey protein films. *Food Science and Technology* 29(2): 420-430.
- Zhang, Z., Arrighi, V., Campbell, L., Lonchamp, J. and Euston, S. R. 2016. Properties of partially denatured whey protein products: Formation and Characterization of structure. *Food Hydrocolloids* 52: 95-105.