

Milk – a review under its synthesis, composition, and quality assurance in dairy industry

¹Andrei C. Grădinaru, ^{1,2}Șteofil Creangă, ¹Gheorghe Solcan

¹ University of Agricultural Sciences and Veterinary Medicine of Iași, Iași, Romania; ² Research and Development Station for Bovine Iași, Iași, Romania.

Abstract. The aim of this paper is to briefly present some literature data with respect to histochemical mechanisms of milk synthesis, its composition in human useful active principles, and its quality assurance throughout its processing. Milk is one of the main foodstuffs consumed by humans, its synthesis at the udder's level based on synthesis and selective filtration processes using a preformed biological material which is circulated through circulatory system. Milk nutritional value is certainly higher than its each component value, and even the necessary components for milk elaboration are coming from blood, between these biological fluids are significant compositional differences. Milk contamination risks are various, from those physicals (foreign subjects such as manure, feed, dust, pieces of broken glass, strands of hair, wood, plastic or metal chips), to chemicals (antibiotic, hormone, pesticides, detergent, or heavy metal residues), to those microbiological ones (germs, somatic cells). In foodstuff industry, and particularly in that of dairy products, the hazard analysis and critical control points (HACCP) concept guarantees foods' quality due to their entire chain supervision, "from farm to fork".

Key Words: milk synthesis, composition, and quality; HACCP.

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Corresponding Authors: A. C. Grădinaru, email: andre_gradinaru@yahoo.com

Introduction

Milk is one of the main foodstuffs consumed by humans, and an important starting base in different human productive activities (Robinson 1994).

Cattles are the primary source of milk in the most word's areas, except the Indian subcontinent and Egypt, Mediterranean regions, Middle East parts and some Africa's areas, where buffalos, sheep and goats have an important contribution on national's milk productions. Camels are used in the moistureless Northern and Eastern Africa's desert, and Middle East. Other species used to get milk are the reindeer in Lapland, and the mare in Central Asia (Tamime 2006).

Milk composition varies depending on species, geographic position, and newborn needs. Milk is as enriched in its compounds as newborn requirements for a quick gain in weight are; the milk sugar proportion is related to the brain development, and milk fats are in a higher content in animals located in cold areas (Maloș et al 2002). Except its ferrum, cooper and C vitamin contents, milk could be considered a complet foodstuff for humans nutrition needs (Banu (a) 2002, Rusu 2005).

The average human requirements for milk per year are estimated within 240 to 300 liters per capita, or minimum 0.5 liters a day or 180 liters per capita, including the processed milk. But the world average milk consumption is by 107 liters per capita, which varies within 380 liters in Europe and 280 liters in Northern America and Oceania, to 50 liters in Southern Asia and 20 liters in Eastern Asia. However, these amounts are constantly changing as a consequence of migration and tourism (Stoicescu 2008). The European milk consumption is higher

in Scandinavian countries (Norway, Sweden, Finland) than in France, Germany, Italy or Greece, where the warm climate lead to milk processing in keeping quality products.

The aim of this paper is to briefly present some literature data with respect to histochemical mechanisms of milk synthesis, its composition in human useful active principles, and its quality assurance throughout its processing.

Histochemical mechanisms of milk synthesis and secretion

The mammalian females are different from those of other species by their mammary gland and its lactating function. These females secrete a white liquid, slightly sweet tasting, knowing as "milk".

Their mammary gland is an apocrine type one, consisting of a fibrous capsule related to abdominal tunic, from where septums are detaching in order to divide the tissue in compartments (quarters, lobes, lobules) (Cotea 2005).

The mammary glandular alveola or the mammary acinus is the morphological and functional unit of mammary parenchyma. This is internally lined by a layer of epithelial cells (alveolar cells, secretory cells, "lacto"- cells) which are active parties of milk making. But milk excretion couldn't be possible without the myoepithelial cells which are associated as a web on the mammary acinus structure, on their contraction the ducts being open and milk passing into gland cistern (~400 ml).

At the udder's level, milk is prepared on the basis of synthesis and selective filtration processes, using a preformed biological

material which is circulated through circulatory system. The alveoli take a part of milk components from blood (water, mineral salts, glucose, amino acids, triglycerides, a part of albumins and globulins, vitamins and other components), which excrete them in the alveoli lumen without any changes. But for the vast majority of lactose, 40% of fats, and 90% of proteins, the syntheses take place at the alveoli level using specific cellular structures. Not only a preformed material is vehiculated through the systemic blood flow; the control of mammary gland development itself couldn't be possible without signaling molecules or "hormones". Even the activity of the alveolar cells is stimulated by prolactin hormone, and the one of the myoepithelial cells, by oxytocin hormone.

The rate of milk synthesis is slowed-down by the accumulation of secreted milk, which increase the pressure in the alveoli. Therefore, as more frequent milk ejection is, the pressure in udder will be decreased and the rate of milk synthesis will be increased. For a daily production of 20 liters of milk, through the cows mammary gland have to be circulated 10,000 liters of blood, 500 liters of blood for each liter of milk being assigned (Şindilar 1998).

Milk precursors pass from blood into extracellular matrix of capillaries and epithelial cells, from where they are directly taken through the baso-lateral membrane of epithelial cells. Inside the epithelial cells, these precursors either are used in biochemical reactions of final components syntheses, or are as such carried and secreted.

Therefore:

A. Amino acids are absorbed from the extracellular matrix and at the polyribosomes level are covalently bonded to form specific proteins. Each polyribosome is crossed by a 2 nm thick messenger RNA molecule, which passes through each ribosome between its small and large subunits. After protein syntheses, the polyribosomal chain is break, the messenger RNA unfold, and the ribosomes disperses in cytoplasm. Polyribosomes can be free on cytoplasm, synthesizing the own or the cell structure proteins (e.g. membrane proteins, membrane enzymes), or attached on the endoplasmic reticulum and forming such a called – rough endoplasmic reticulum, which is acting in order to synthesize specific export proteins, such as caseins, beta-lactoglobulin, alpha-lactalbumin, or other export proteins, such as enzymes, hormones or antibodies which can be transmitted through milk.

The new synthesized proteins are passed from the rough endoplasmic reticulum to Golgi apparatus, where are prepared for moving out of the cell. Casein is secreted by Golgi apparatus as micelle, formed by its association with calcium and phosphorus. The protein biosynthesis stages are similar to any protein:

- the transcription of the genetic message encoded in DNA by a molecule of messenger RNA. Firstly, the RNA polymerase enzyme synthesize a precursor messenger RNA which contain the exons and introns corresponding sequences of the transcribed gene. This pre-messenger RNA became "mature", and after it will finish its duty it is the subject of enzymatic hydrolysis and depolymerization ((Creighton 1999; Passarge 2001).
- the cytoplasmic amino acids activation through their reaction with Adenosine triphosphate (ATP), and attachment at the transfer RNA, this molecule being specific for each amino acid.
- the genetic message translation at ribosomal level. The messenger RNA molecule contain sequences of three nucleotides

for each amino acid, known as codons. A specific trinucleotide anticodon located at a transfer RNA molecule, recognize a specific codon; the amino acid – transfer RNA complex changes its position according to the position of each codon in the messenger RNA molecule (Lehninger 2004, 2005; Tămaş *et al* 1981).

B. Milk sugar. In order to synthesize each lactose molecule are required the glucose molecules, one being used as it is, and another one being converted in Uridine-Diphosphate Glucose (UDP), and then in Uridine Diphosphate Galactose (UDG). The Uridine Diphosphate accumulation in Golgi apparatus lumen could be an inhibitor in lactose synthesis; to prevent this fact, this molecule is quickly hydrolysed by Nucleoside Diphosphatase enzyme (NDP enzyme) in Uridin Monophosphat (UMP) and inorganic phosphorus.

The lactose synthesis reaction is catalyzed by lactose-synthetase enzyme, being considered in one sense, thereby the dissolution of lactose molecule by hydrolysis at glucose and galactose it is no longer possible. Moreover, the lactose increased levels in Golgi apparatus can not inhibit its own synthesis (<http://www.classes.ansci.illinois.edu/ansc438/Milkcompsynth/milksynthlactosesynth>).

C. Triglycerides (triacylglycerols or triacylglycerides) are synthesized using fatty acids and glycerols. They represent the main component of milk fat, the palmitic (C16:0) and oleic (C18:1) acids sharing a large quota on their composition.

The used fatty acids for milk fat synthesis can be derived either from blood lipids or from "de novo" synthesis inside the mammary epithelial cells starting from blood absorbed precursors. More than 80% of blood fatty acids are originated from diet, with variations depending on lactation stage and milk production. The acetate, beta hydroxybutyrate, glycerol or monoacylglycerols are precursors used at milk triglyceride synthesis at smooth endoplasmic reticulum level. The rumen produced acetate and beta hydroxybutyrate are used at short or medium chains fatty acids, almost 17 to 45% of cow's milk fat coming from acetate, and 8 to 25%, from butyrate. The lack of fiber in their diet decrease acetate synthesis in rumen, the obtained milk being with a lower fat content (2-2.5%) (Georgescu 2007, Tămaş *et al* 1981). In the first stage of milk fat synthesis, there are formed small drops of fat, which will progressively merge into a bigger drop of fat. This one will force the apical pole membrane and will be released as a fat globule covered by a membrane originating from the apical pole of the epithelial cells (<http://www.classes.ansci.illinois.edu/ansc438/Milkcompsynth/milksynthfatoverall>).

D. Some of milk components are directly taken from blood, after they passed without any changing through the epithelial cells barrier. Thus, immunoglobulins, serum albumin, minerals, and other small xenobiotics can pass into milk content.

Milk compositional features

Even the necessary components for milk elaboration are coming from blood, between these biological fluids are significant compositional differences.

Cotea (2005) notes milk contain ninefold more fat, ninetyfold more sugar, fivefold more potassium, thirteenfold more calcium, and tenfold more phosphorus than blood, which demonstrate the mammary gland as a complex biological laboratory capable to synthesize new components from those existing in blood. Alais *et al* 1985 investigated some compositional differences between milk and blood plasma, their results being presented in table 1.

Table 1. The composition of the cow's milk and blood plasma(g/l)

Components	Milk	Blood plasma
Fat	35	3
Lactose	49	0
Caseins	27	0
α-lactalbumin and β-lactoglobulin	4	0
Albumin and globulin	1.5	75
Citric acid	2	0
Chlorides	1.6	6
Phosphates	2.5	0.3

In pathological milk, as a consequence of synthesis activity decreasing at mammary level, and of milk content increasing in blood components, lactose, fat and casein contents will decrease, and the albumin, globulin and chlorides contents will increase. The fat content of pathological milk will rarely increase, more probably as a consequence of total amount of milk sudded decreasing (Alais et al 1985, Grădinaru et al 2007).

Milk nutritional value is certainly higher than its each component value. The milk chemical composition of some mammalian animals is shown in table 2.

Table 2. The milk chemical composition of some mammalian animals (g%)
(<http://www.classes.aces.uiuc.edu/AnSci308/milkcomp.html>)

Species	Fats	Proteins	Lactose	Minerals	Total solids	Water
Cow (Holstein)	3.5	3.1	4.9	0.7	12.2	87.8
Cow (Jersey)	5.5	3.9	4.9	0.7	15	85
Sheep	5.3	5.5	4.6	0.9	16.3	83.7
Goat	3.5	3.1	4.6	0.79	12	88
Woman	4.5	1.1	6.8	0.2	12.6	87.4
Mare	1.6	2.7	6.1	0.51	10.9	89.1
Donkey	1.2	1.7	6.9	0.45	10.2	89.8
Buffalo	10.4	5.9	4.3	0.8	21.4	78.6
Sow	8.2	5.8	4.8	0.63	19.4	80.6
Bison	1.7	4.8	5.7	0.96	13.2	86.8
Camel	4.9	3.7	5.1	0.7	14.4	85.6
Reindeer	22.5	10.3	2.5	1.4	36.7	63.3

Milk water represents an important share of milk content, being important more for its solvent properties and good vehicle of endogene or exogene milk constituents. Milk fats are the most variable compounds, being under the influence of specie, breed, lactation stage, feeding, season, and health condition (Rotaru et al 1997, Şindilar 1998). Milk nitrogen compounds can be proteics or non-proteics, in a ratio of 95/5. Milk and dairy products represent an important source of proteins at superior nutritive value due to their content in all essential amino acids. Milk carbohydrates are consisted by lactose and other sugars in a low

amount (such as glucose, galactose). They are the second largest compound in milk composition, considering water the first one (Banu (b) 2002; Iurcă 1998; Mihaiu & Mihaiu 1998). Despite of their large number, milk enzymes are in small concentrations, being located at lipoprotein membrane of fat globules, at casein micelle, or in solution. Milk is a foodstuff which contain in balanced proportions almost all of the liposoluble (A, D, E, K) and hydrosoluble (B, C, P) vitamins; however, milk remains a poor source of C, PP, and folic acid vitamins. Milk pigments are endogenous and exogenous. Lactoflavin and riboflavin color milk in greenish-blue and yellowish-beige, respectively, being the most important pigments of animal origin. The exogenous pigments are taken from feed or as a result of milk contamination with carrying-pigments microorganisms. The most important exogenous pigment is carotene; beside this one, milk can contain xanthophyll and chlorophyll. In fresh milked milk, gases such carbon dioxide, nitrogen and oxygen are in higher amounts than ammonia and hydrogen sulphide. Milk minerals are represented either by soluble or insoluble salts (chlorides, phosphates, citrates), or by bounded-forms by milk proteins, especially by caseins. Over unity calcium-phosphorus ratio in milk (120:90 mg/100 ml) is considered one of the most favourable growing and bones developing factors. Milk is also an important source of cobalt, iodine, zinc, less important for chromium and nickel, and even a poor one for manganese, ferrum and cooper. An exclusive feeding with milk may lead to iron deficiency anemia due to its inadequate potential for hemoglobin synthesis (Banu (b) 2002; Ciotău 2006; <http://www.thedairysite.com>).

Milk cells comes from blood and mammary gland, and they are known as "somatic milk cells" (Popa & Popescu 1973, Seiciu & Voiculescu 1997). Milk somatic cells are classified using a morphological and functional criteria (Rotaru & Ognean 1998), in:

- polymorphonuclear leukocytes, especially neutrophils with microbial phagocytic activities;
- monocyte-macrophage cells, which have phagocytic action and an important role in pathogens-lymphocyte reaction preparing.
- lymphocytes, particularly T lymphocytes, which release lymphokines with role in leukocyte influx initiation by chimiotactism.
- epithelial cells derived from mammary glandular epithelium exfoliation, without any role in milk composition.

Milk somatic cells presence is a veritable indicator of milk quality permanently supervised in the last years. In a "healthy" milk, their number is within 50,000 to 100,000 /ml, depending on stabulation/grazing period, or mammary gland diseases. The cytological examination of milk became mandatory in every milk processing unit; even somatic cells "per se" do not directly influence the consumer health, their presence in milk represent an important indicator of mammary gland functional status, being asociated in many cases with microorganism increased levels (Allison et al 1985; Grădinaru et al 2009; Pantoja et al 2009; Ruegg & Tabone 2000; Van Schaik 2002).

Quality assurance in dairy industry

Dairy products control under their sensory, phisico-chemical, and microbiological features is inefficient due to the impossibility to check all the products. Moreover, when are discovered inadequates, in many cases it is too late to take adequate economical actions, usually the quality responsible staff of milk processing unit deciding to send the entire lot to reprocessing,

or to change its destination, or to destroy it (Rotaru & Moraru 1997). Thus, in dairy industry it might be recorded time and financial losses. But, these can be avoided if key elements of dairy products manufacturing process are permanently under control, many corrective measures being applied on time. Since 1993, the 93/43 Council Directive considering the hygiene of foodstuffs, stipulated the necessity of a unitary system of foodstuffs quality checking, whose effect to be the consumer trust increasing in food safety. Subsequently, many countries legislations stipulated the HACCP introduction and application in foodstuffs' network, reinforcing Codex Alimentarius recommendations on quality management based on Hazard Analysis on Critical Control Points. If milk is considered, it is well-known this is obtained in an environment with increased risks for contamination and pollution. In the same time, milk has a tendency to perish, being exposed on various risks after milking, during its transportation to processing units, or even during its processing. However, a "decision tree" usage permit to appreciate if a potential physical, chemical, or biological risk indeed represent a risk for the consumer or the manufacturing process. After risks evaluation, it is mandatory to establish preventive measures in order to ensure their removal or decreasing (Goncearov & Petcu 2003, Goncearov *et al* 2004).

Milk contamination risks are various, from those physicals (foreign subjects such as manure, feed, dust, pieces of broken glass, strands of hair, wood, plastic or metal chips), to chemicals (antibiotic, hormone, pesticides, detergent, or heavy metal residues), to those microbiological ones (germs, somatic cells). In HACCP application, there are two kind of critical control points: CCP1 – which ensure the risk removal, and CCP2 – which reduce the risk, but does not completely remove it, remaining at an acceptable level. On the other hand, the control points (CPs) are paths of control applied on different technological stages which are not criticals due to their decreased risk (Georgescu 2005, Lawley *et al* 2008).

In foodstuff industry, and particularly in that of dairy products, the HACCP concept guarantees foods' quality due to their entire chain supervision, "from farm to fork". This system significantly contributes on food quality improving, rather accentuating the needs for anticipation and prevention, than for final products inspection and control (Banu 2003; Brădăţan 2007; Mencinicopschi & Raba 2005; Solcan 2006).

Conclusions

The modern concept of "milk" include all the nutritive, structural, technological, and commercial criteria. There is not considered for human nutrition milk from sick animals, colored, dirty, smelly, or colostrum milk, which contain physical, chemical, or microbiological risks.

In order to ensure a proper quality of milk and its derived products, HACCP was proposed and applied in many countries as a systematic preventive approach, and an efficient path to design measurements to reduce risks to a safe level.

References

- Allison JRD. Antibiotic residues in milk. *British Veterinary Journal* 1985;141:121-124.
- Banu C (a). The manual of food industry engineer. 1, Tehnical Publishing House, Bucureşti, 2002. [in Romanian]
- Banu C (b). Food chemistry. Agir Publishing House, Bucureşti, 2002. [in Romanian]
- Banu C. Principles of food law. Agir Publishing House, Bucureşti, 2003. [in Romanian]
- Brădăţan Gh. Food safety, Ion Ionescu de la Brad Publishing House, Iaşi, 2007. [in Romanian]
- Ciotău C. Contributions to appreciate the quality and hygienic condition of some types of milk powder produced by Rarăul, Câmpulung Moldovenesc. PhD thesis, Veterinary Faculty of Iaşi, 2006. [in Romanian]
- Cotea C. Special Histology. Tehnopress Publishing House, Iaşi, 2005 [in Romanian]
- Council Directive 93/43, The hygiene of foodstuffs. Official Journal of European Communities, 1993;L175:1-11.
- Creighton TE, Molecular biology, Vol.1-4, John Wiley & Sons, USA, 1999.
- Georgescu Gh. The book of milk producers and processors. Knowledge of milk processing. 4, Ceres Publishing House, Bucureşti, 2005. [in Romanian]
- Georgescu Gh. The book of milk producers and processors. The exploitation of dairy cattle. 2, Ceres Publishing House, Bucureşti, 2007. [in Romanian]
- Goncearov M, Petcu C. General principles of HACCP implementation in food processing units. *Revista Română de Medicină Veterinară* 2003;13(2):118-125. [in Romanian]
- Goncearov M, Petcu C, Antoniu S. Hazard analysis critical control points - a modern concept regarding food quality and safety. *Scientific Papers: Veterinary Medicine, Timişoara* 2004;37:868-872. [in Romanian]
- Grădinaru AC, Havriştiuc S, Popescu O. Evaluation of the milk antibiotic residues incidence and their influence upon some milk quality indexes as part of the HACCP principles implementation. *Scientific Papers: Veterinary Medicine, Iaşi* 2009;52(11):947-949. [in Romanian]
- Grădinaru AC, Lazăr R, Popescu O, Boişteanu P, Dohotariu L. Evaluation of the open market milk quality by measurement of some physico-chemical parameters. *Scientific Papers: Veterinary Medicine, Iaşi* 2007;50(9):173-176. [in Romanian]
- Iurcă IM, Milk and milk products. I.C.P.I.A.F. Publishing House, Cluj-Napoca, 1998. [in Romanian]
- Lawley R, Curtis L, Davis J. Food safety hazard guidebook. Royal Society of Chemistry Publishing, Cambridge, 2008.
- Lees M. Food authenticity and traceability. Woodhead Publishing Ltd., Cambridge, UK and CRC Press, Boca Raton, 2003.
- Lehninger AL. Biochemistry. 4,5. W.H.Freeman & Company, New York, 2004, 2005.
- Maloş G, Maloş IG, Diaconescu A, Ianiţchi D. Milk processing and cheese. UASMV Publishing House, Bucureşti, 2002. [in Romanian]
- Mencinicopschi Gh, Raba DN. Food security-Authenticity and traceability. Mirton Publishing House, Timişoara, 2005. [in Romanian]
- Mihaiu M, Mihaiu R. Milk and its quality control. Risoprint Publishing House, Cluj – Napoca, 1998. [in Romanian]
- Pantoja JCF, Reinemann DJ, Ruegg PL. Associations among milk quality indicators in raw bulk milk. *Journal of Dairy Science* 2009;92(10):4978-4987.
- Passarge E., Color Atlas of Genetics, 2nd Edition, Thieme, New York, 2001.

- Popa G, Popescu N. Guidelines for the control of food of animal origin. Ceres Publishing House, București, 1973. [in Romanian]
- Robinson RK. Modern dairy technology. 1,2. Elsevier, New York, 1994.
- Rotaru G, Moraru C. HACCP-Hazard Analysis Critical Control Point. Academica Publishing House, Galați, 1997. [in Romanian]
- Rotaru O, Guș C, Mihaiu M. Introduction in veterinary control of animal products hygiene. Genesis Publishing House, Cluj – Napoca, 1997. [in Romanian]
- Rotaru O, Ognean L. The morphology and physiology of milk cell population. Casa Cărții de Știință Publishing House, Cluj – Napoca, 1998. [in Romanian]
- Ruegg PL, Tabone TJ. The relationship between antibiotic residue violations and somatic cell counts in Wisconsin Dairy Herds. *Journal of Dairy Science* 2000;83(12):2805-2809.
- Rusu V. Research on technological, quality, and of hygiene aspects of cheeses produced in the mountains of Northern Moldova. PhD thesis, Veterinary Faculty of Iași, 2005. [in Romanian]
- Seiciu F, Voiculescu S. The mammary gland-morphology and pathology. România de Măine Publishing House, București, 1997. [in Romanian]
- Solcan G. Herd health and production management system in dairy cows. *Cercetări Agronomice în Moldova* 2006;2(126):71-74.
- Stoicescu AV. Monitoring of chemical residues in some foods of animal origin. PhD thesis, Veterinary Faculty of București, 2008. [in Romanian]
- Șindilar E. The hygienic control of animal origin products and by-products. 2, Moldogrup Publishing House, Iași, 1998. [in Romanian]
- Tamime AY. Brined cheeses. Blackwell Publishing, Ames, Iowa, 2006.
- Van Schaik G, Lotem M, Schukken YH. Trends in somatic cell counts, bacterial counts, and antibiotic residue violations in New York State during 1999 – 2000. *Journal of Dairy Science* 2002;85(4):782-789. <http://www.classes.aces.uiuc.edu/AnSci308/milkcomp.html>.
- <http://www.classes.ansci.illinois.edu/ansc438/Milkcompsynth/milksynthfatoverall>.
- <http://www.classes.ansci.illinois.edu/ansc438/Milkcompsynth/milksynthlactosesynth>.
- <http://www.thedairysite.com>.

Authors

- Andrei C. Grădinaru, Department of Animal Genetics, Faculty of Animal Sciences, Aleea M. Sadoveanu 8, 700489, Iași, Romania, EU, email: andre_gradinaru@yahoo.com.
- Șteofil Creangă, Department of Animal Genetics, Faculty of Animal Sciences, Aleea M. Sadoveanu 8, 700489, Iași, Romania, EU; Research and Development Station for Bovine Iași, Șos. Iași-Ungheni 9, 707252, Iași, Romania, EU, email: creanga62@yahoo.com.
- Gheorghe Solcan, Department of Internal Medicine, Faculty of Veterinary Medicine, Aleea M. Sadoveanu 8, 700489, Iași, Romania, EU, email: gsolcan@yahoo.com.

Citation Grădinaru AC, Creangă Ș, Solcan G. Milk – a review under its synthesis, composition, and quality assurance in dairy industry. *HVM Bioflux* 2015;7(3):173-177.

Editor Stefan C. Vesa

Received 15 June 2015

Accepted 28 June 2015

Published Online 2 July 2015

Funding None reported

**Conflicts/
Competing
Interests** None reported