12 General Conclusions

Considering the properties to define the qualification of foods, quality and risk factors could be studied within a unique system, i.e. the feed chain in milk production which strongly affects the quality and safety of milk. Within the quality of milk, the improvement of iodine and selenium content, the adulteration of feed/milk with melamine and the analysis of iodine in milk, are analysed in this doctoral thesis.

Milk is recognized as an important food for preventing iodine deficiency disorders. In our trial, the concentration and total excretion of iodine in cow's milk increased linearly with iodine intake. The carry over of iodine into milk was about 15% of the ingested in the basal diet (1.7 mg/kg DM), while it was almost 26% in cows fed iodine supplements (basal diet + 23 or 46 mg I/d). The stage of lactation/milk yield did not affect the concentration of iodine in milk. When cows were fed 65 mg/d of iodine, the milk iodine content was about 600 µg/L. Considering a normal consumption of milk (about 150 ml/head/d), the latter value of milk iodine concentration should guarantee a considerable amount of iodine for human nutrition, particularly for children and lactating women. During cheese making, the iodine was transferred mainly (~85%) in the whey fraction and the enrichment factor of iodine in cheese was 1.7-fold, thus, also dairy products could be a valid source of iodine for human.

Although discussions on the recommended daily allowance (RDA) for selenium are still open, the milk could be an important source of selenium for human nutrition. In our trial, using sodium selenite, the concentration and total excretion of selenium in milk increased linearly only in the first experiment: this probably due to the low concentration of selenium in the basal diet. The average concentration of selenium in milk ranged from 20 to 24 µg/L when cows were fed from 0.25 to 0.5 mg/kg DM of selenium. The carry over of selenium in milk in experiment 1 was 26% for lower selenium diet (0.08 mg/kg DM), and about 9.3% for diet close to 0.3 mg/kg DM, that is the content of selenium in the diet of lactating dairy cows suggested by NRC. The transfer efficiency of sodium selenite from feed to milk was quite low, as expected. The stage of lactation/milk yield affected the concentration of selenium in milk (to higher milk yield corresponded a lower selenium concentration). During cheese-making, about 75% of total selenium from milk was transferred into cheese, while the enrichment factor of selenium in cheese ranged from 5 to 7-fold. Since the high enrichment factor in cheese, selenium intake in human should be more effective compared to milk consumption for the coverage of selenium RDA. Further studies should be addressed to validate the best selenium source as feed additive, also considering the ratio between 'human health profit' and 'farming cost and animals health'. Moreover, literature data do not completely explain the different bioavailability of selenium from milk produced by cows fed sodium selenite or selenized-yeast.

The health breakout concerning contamination with melamine of milk in China and pet-food in the United States of America raised questions about the safety of milk produced by cows fed melamine-tainted feed. In our trial on melamine we confirmed the pathway for the transmission of melamine from feed to milk and its rapid excretion either

at high (2500 mg/kg DM) or low (2.5 mg/kg DM) level of melamine ingestion. The concentration and total output (the latter measured during 7 days after single ingestion) of melamine in milk increased ($P<0.001$, cubical effect) with ingestion; when cows were fed 2.5, 25, 250 and 2500 mg/kg DM of melamine, the milk contained 0.02, 0.86, 2.93 and 35.10 mg/Kg fluid milk, respectively. The average transfer of melamine from feed to milk was 4.4%, however further studies could be addressed to better understand the excretion pattern of melamine into milk. Melamine did not affect milk crude proteins, crude fats and lactose contents. On the contrary, the milk urea nitrogen decreased linearly with increasing melamine ingestion. The melamine disappeared from milk within 7 days from a single oral dose, and the rate of clearance decreased linearly from the high to the low level of melamine ingestion. During the cheese-making melamine content of milk did not affect the process and melamine mainly transferred (~85%) from milk to whey. The mass balance data suggested that melamine could be subjected to loss or bio-transformation during cheese making and/or ripening. The European safety limit of 1 and 2.5 mg/kg of melamine for children food and general food/feed, respectively, appears valid to guarantee the safety of consumers. The milk produced in our trial was higher than those levels when were cows fed at least 25 mg melamine / kg of DM. The enrichment factor of milk (~8.5) and whey (14-17), makes the direct (infant formula) or indirect (as feed/food additives or ingredients) ingestion of melamine-tainted dry milk and whey to be a serious health hazard. Whereas, since the low transfer of melamine from milk to cheese, only the ingestion of cheese derived from cows fed at least 250 mg/kg DM should represent a human health risk.

For an efficient management of feed fortification with iodine, farm, milling and dairy industries could need sound and rapid analytical support. The current official methods for the iodine analysis are based on the ICP-MS determination after the element extraction with tetra methyl-ammonium hydroxide (TMAH). Unfortunately, the extraction with TMAH lasts 3 hours and the chemicals used are quite expensive. In our study, using the bottom-up approach, we compared uncertainty and efficiency of the 0.5% ammonia solution (pN in open system) and the TMAH (pT in closed system; official method EN15111:2007) extraction of iodine in whole and skimmed raw milk (at two levels of iodine concentration). The time of extraction was dramatically quicker for pN (2.5 *vs.* 180 min). The expanded uncertainties were quite similar between methods within the level of iodine concentration, and numerically ranged from 0.015 to 0.03. The contribution of precision on uncertainty in pT was almost 2-fold higher than that of the pN method. The difference in relative precision contribution could be explained by the use of disposable materials in the pN procedure. The pN method was performed in skimmed milk for improving the extraction capability of 0.5% ammonia solution. The analysis of total iodine in skimmed milk by pN method was about 5% lower than the value of total iodine in whole milk measured by the pT method. Therefore, a rapid de-fatting step could be appropriate for an accurate determination of total iodine in milk. Considering the efficiency, the precision, the short time of extraction and the lower costs of analysis (0.0034 *vs.* 0.8 €/sample), the pN procedure appeared a valid alternative to the official

EN1511:2007 method for the total iodine determination in milk in laboratories equipped with ICP-MS.

The bioavailable form of iodine is iodide. In our study settled a chromatographic method for evaluating the inorganic form of iodine in milk produced by cows fed iodine supplements. Iodine was efficiently extracted from raw milk by using 0.5% ammonia solution in an open system microwave digestion. Samples were analysed with a HPLC ionic column coupled in-line with an ICP-MS. The percentage of iodide compared to the total iodine content was about 75% in all milk samples (non- and fortified milk), and the level of total iodine content did not influence the percentage of iodide into milk. Iodate was never detected in all samples. Our procedure offered sensitive and rapid separation of the inorganic species, iodate and iodide, in skimmed raw milk.