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Research Article

Effect of Emulsifying Salt Type on Physiochemical Properties of Processed Cheese as Well as Genetic and Biochemical Changes in Male Mice

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Abstract

The present investigation was an attempt to evaluate processed cheese samples made by using two types of emulsifying salt. First treatment (T_1) was prepared by using tri-sodium citrate (cheese 1), while second treatment (T_2) achieved by using sodium polyphosphate (cheese 2). All cheese samples were analyzed for their physiochemical properties and organoleptic characteristics. On the other side, cytogenetics evaluation including chromosomal aberrations in somatic and germ cells, DNA damage and micronucleus tests were carried out in male mice treated with cheese 1 and 2. Moreover, the effect of cheese 1 and 2 on liver function and kidney function in male mice were also studied. The results revealed that cheese 1 possessed high values of meltability and oil separation and low values of penetration compared with cheese 2. The genotoxicity study revealed that male mice treated with cheese 1 and 2 expressed significant increment in the chromosomal aberrations, DNA damage and micronucleus formation compared to control mice. In addition, levels of ALT, AST and ALP as well as creatinine, urea and uric acid were markedly increased in male mice treated with cheese 1 or 2 compared with control mice. However, cheese 2 was more effective in increase the genetic toxicity than cheese 1.The present results revealed that use of tri-sodium citrate and sodium polyphosphate in cheese processing increased the oxidative stress in male mice that increased the toxicity response on genetic materials, liver and kidney functions. So, an urgent demand for searching new materials used as emulsifying agents is still need.

Key words: Processed cheese, emulsifying salts, physicochemical properties, chromosomal aberrations, DNA damage, liver and kidney functions

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Processed cheese is generally, produced by blending natural cheese of various ages and degrees of maturity in the presence of emulsifying salts, stabilizer and other ingredients followed by cooking to form a homogeneous product (Mihaela *et al*., 2013). Emulsifying salts are the main ingredient in the processed cheese. In their absence, processing would lead to forming of a heterogeneous, gummy and product (Guinee, 2007). The two main roles of emulsifying salts are chelation of calcium (that aids in breaking the calcium-phosphate cross-linked protein network present in natural cheese) and pH adjustment (Chandan and Kapoor, 2011). The types most commonly used, are sodium citrates, sodium hydrogen orthophosphates, sodium polyphosphates, and sodium aluminum phosphates (Shimp, 1985). Tri-sodium citrate and disodium phosphate were reported to common for used and produce the most pronounced melting effects. Sodium citrate induced significant melting only at the highest tested concentration (3.0%) (Pal, 2002). Due to this particular pH increase, the calcium-masking ability of the emulsifying salts is increased by the negative charges on the para-caseinate, thus promoting an increase in hydration and solubility of para-casein and the formation of a stable product (Mulsow *et al*., 2008).

From the health concept, the type and concentrations of emulsifier salts had closely effect. The dietary intake of phosphate concentration is important concept not just for persons with renal disease, but for the public as well. Phosphate additives in food may harm the health of persons with normal renal function (Sullivan *et al*., 2009). It was recognized in patients with renal disease. High serum phosphate concentration is a major risk factor for elevated cardiovascular and overall mortality (Block *et al*., 2004; Kestenbaum *et al*., 2005). Furthermore, dietary phosphate restriction has been a standard recommendation for patients with chronic renal failure KDIGO (2009).

Therefore, the goal of this research is to evaluate some emulsifying salts (pure tri-sodium citrate and commercial sodium polyphosphate) that already used in processed cheese manufacture. Studying the physiochemical properties of resultant cheese and potential genetic and biochemical toxicity in male mice was also a target.

MATERIALS AND METHODS

First part (Concerning with cheese sample)

Materials:

- Ras cheese (one month old) was obtained from Arabic Food Industrial Co. (Dometty), 6th October City, Egypt
- Matured Cheddar cheese (8 months old) was obtained from International Dairy and Food Co. (Green Land), 10th Ramadan City, Egypt
- Low-heat- skim milk powder (SMP) and butter were obtained from local market (procured from Irish Dairy Board, Hawse, Ireland)
- **Emulsifying salts (ES):** Tri sodium citrate (pure laboratory trade mark) and commercial mixture of sodium polyphosphate. Table 1 revealed the chemical composition of the ingredients that used in the manufacturing of processed cheese

The quantity of the ingredients (kg/100 kg) of the blend that used in manufacture of processed cheese spreads is as follow: Cheddar cheese was12.80%, ras cheese was38.44%, S.M.P was 5.12%, butter was 10.26%, emulsifying salt was 3% and water was 30. 88%.

Experimental design: Three batches of Processed Cheese (PCs) sample of each treatment were prepared by applying the traditional method as mentioned by Abdel-Hamid *et al*. (2000).

Treatments: First cheese was prepared by using 3% pure tri-sodium citrate (T_1), while second cheese was prepared by using 3% commercial polyphosphate (T_2).

Methods of analysis

Chemical composition of processed cheese: The resultants PCs were fresh checked for their Total Solids (TS), Total Protein (TP), Soluble Nitrogen (SN) and fat contents according to AOAC (2007). Ash content was determined as mentioned by IDF (1964). The pH value was determined using a digital laboratory pH meter (HI 93 1400, Hanna instruments).

Physicochemical properties: Melting quality was estimated by the method of Savello *et al*. (1989). Oil separation index was determined according to Thomas *et al*. (1980) Penetration was also estimated using penetrometer Cochler Co. INC., USA.

Color parameters: Color was measured using Hunter Colorimeter model D2s A-2 (Hunter, 1975). Tri-stimulus values of the color namely L, a and b were measured using the corresponding button on the colorimeter.

Where:

L = Represents darkness from black (0) to white (100)
a = Represents color ranging from red (+) to green (-)
b = Represents color ranging from yellow (+) to blue (-)

Table 1: Chemical composition of ingredients used in manufacture of processed cheese

	Ingredients			
Compositions	Cheddar cheese	Ras cheese	Skim milk powder	Butter
Total solids (%)	66.01	54.62	96.00	84.00
Fat/DM	52.95	45.00	1.04	97.61
Total protein (%)	25.44	22.22	37.13	ND
Total carbohydrate (%)	0.10	1.60	47.43	ND

Sensory properties: Sensory properties of the processed cheese were evaluated by panel test by the staff members at the Dairy Department of National Research Center, Cairo, Egypt, using to the graphical descriptor scale limits scheme.

Statistical analysis: Statistical analysis was performed according to the User's Guide given by SAS (2004) using Least Significant Differences (LSD).

Second part (concerning Cytogenetic study)

Experimental design: Adult male's albino mice each weighting (30 g) served as experimental animals. The mice were housed in plastic cages at an environmentally controlled room (constant temperature 25-27°C) with 12 h. Light/dark cycle for one week prior to starting the experiment was applied and they were provided with water and *libitum*. Mice was divided into three groups, each group contented from 10 animals. First group served as control which fed standard diet. Second group; animals were fed cheese made by sodium citrate for 30 days (T_1). Third group; animals were fed with cheese made by sodium polyphosphate (T_2).

All animals were treated for 30 consecutive days and after 24 h from the last treatment; animals were sacrificed by cervical dislocation for studying cytogenetic analysis and biochemical study.

Chromosomal preparation in bone marrow cells: Chromosomes from bone marrow cells were prepared according to the method of Hsu and Patton (1969). Mice were injected i.p. with colchicine's (2.5 mg kg^{-1} b.wt.) and then after 3 h animals were killed by cervical dislocation. The bone marrow cells were collected in phosphate buffer solution (pH 7.2) and centrifuged at 1000 rpm for 2 min. The obtained pellets were mixed in aqueous solution of KCl (0.56%) and left for 30 min at 37°C. The prepared cells were re-centrifuged, and fixed in carnoy solution (methyl:glacial acetic acid, 3:1). Finally slides were air-dried and stained with 10% Giemsa dye for 20 min.

Chromosomal preparation in spermatocytes: Mice testes were collected from the same animals to study the abnormalities in spermatocytes (germ cell) according to Brewen and Preston (1978) with some modifications. Briefly, mice were injected i.p with colchicine's (2.5 mg kg^{-1} b.wt.) after then 3 h later animals were killed by cervical dislocation. The testes were collected in 2.2% sodium citrate solution and minced into pieces with scissors and then centrifuged at 1000 rpm for 2 min. The pellets obtained were mixed in aqueous solution of Na citrate 1.1% and left for 25 min at 37°C. The prepared cells were re-centrifuged, fixed in carnoy solution. Finally, two: three drops of cell suspension were dropped on a clean slide, air-dried and stained with 10% giemsa stain for 25 min. Metaphases (n = 50) were studied per animal scoring different types of aberrations in bone marrow and spermatocytes.

Comet assay: The comet assay was performed under alkaline conditions essentially according to the procedure of Singh (Klaude *et al*., 1996) with minor modification. A freshly prepared suspension of liver cells in 0.75% low melting point agarose (Sigma chemicals) dissolved in phosphate buffered saline (PBS; sigma chemicals) was cast onto microscope slides pre coated with 0.5% normal melting agarose. The cells then lysed for 4 h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% triton×100, 10 mM tris, pH 10. Afterwards, DNA was allowed to Unwind for 40 min in electrophoretic solution consisting of 300 mM NaOH, 1 mM EDTA, pH>15. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73 V cm^{-1} (30 mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 mg mL^{-1} ethidium bromide and covered with cover slips. The slides were examined at 200x magnification fluorescence microscope. Fifty images were randomly selected from each sample and the comet DNA was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells. Each experiment was repeated two times. Percentage of DNA damage was analyzed. The mean value of % DNA in a particular sample was taken as an index of DNA damage in this sample.

Micronucleus test: Bone marrow slides were prepared according to the method described by Krishna and Hayashi (2000). The bone marrow were washed with 1 mL of fetal calf serum and then smeared on clean slides. The slides were left

Table 2: Chemical composition of processed cheese samples prepared by two types of emulsifying salts

Chemical composition (%)	T_1	T_2
TS	45.45	45.76
Fat	24.05	24.07
TP	14.39	14.42
Lactose	3.02	3.05
Ash	4.11	4.27

T_1: Made by organic salt (sodium citrate),T_2: Made by commercial salt (sodium polyphosphate)

Table 3: pH values and sodium and phosphorus contents of processed cheese samples prepared by two types of emulsifying salts

Values	T_1	T_2
pH	5.28[a]	5.80[b]
Na^+ (mg/100g)	1450[a]	1398[b]
P^{+++} (mg/100g)	248[a]	1121[b]

T_1: Made by organic salt (sodium citrate), T_2: Made by commercial salt (sodium polyphosphate)

to air dry and then fixed in methanol for 5 min, followed by staining in 5% Giemsa stain for 5 min then washed in distilled water and mounted. For each animal 1000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

Biochemical analysis

Determination of lipid peroxidation: Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA). Malondialdehyde forms a 1:2 a duct with thiobarbituric acid which can be measured by spectrophotometry. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea *et al*. (1994) in which the thiobarbituric acid reactive substances react with thio-barbituric acid to produce a red colored complex having peak absorbance at 532 nm.

Liver function: Blood samples were collected from each animal in a clear sterile centrifuge tube without anticoagulant obtaining serum for determination of serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and ALP Alkaline phosphatase. The tubes were left at room temperature. After 1 h the tubes centrifuged at 2500 rpm for 10 min the supernatant which is serum, was assayed for (ALT and AST) using ranox kit following Standard method of Reitman and Frankel (1957) and the activity of alkaline phosphatase was measured according to the method of Bowers and McCommb (1966).

Kidney function: Serum was obtained from control and treated mice of each group by centrifugation of blood at 900 rpm for 15 min. The urea and uric acid concentration was determined using modified Berthelot method of Marsh *et al*. (1965) and creatinine concentration by alkaline picture method Standard method of using by Owen *et al*. (1954).

RESULTS AND DISCUSSION

First part

Chemical composition of processed cheese: Table 2 showed the chemical composition of processed cheese samples which prepared by using two types of emulsifying salts (T_1 and T_2). Total solids content of the two treatments was similar as formulations were designed to ensure fixed moisture content. There were no differences between the two treatments in their contents of fat, TP, lactose and ash. Types of emulsifying salts used in processed cheese samples had not been affected the chemical composition parameters. Also, TS% of samples was similar to that found by Suleiman *et al.* (2011), while the results for TP and fat were almost similar to Mihaela *et al.* (2013).

Table 3, represented pH values as well as sodium and phosphorus contents (mg/100 g) of cheese samples. Inorganic emulsifying salt-processed cheese sample had higher pH values than organic emulsifying salt- processed cheese. The pH-values often depending on the raw materials that used in manufacturing of processed cheese. Therefore, it could be notice that sodium citrate, which considered as acidic emulsifier salts; tended to acidity performance than polyphosphate.

Raw materials influences the specific parameters of the melting process, cheese quality and safety of final products (Mihaela *et al*., 2013). The pH value was a good factor which provides useful information on the degree of maturation of raw materials, the possibility to choose the type and the amount of emulsifying salts (Mihaela *et al*., 2013). According to Fox *et al*. (2000), the buffering capacity of sodium salts neither in the pH range nor with an increase in chain size and is practically zero for long-chain phosphates. Fox *et al*. (2000), study agreement with the results obtained by Molins (1991), Guinee *et al*. (2004) and Mulsow *et al.* (2008).

From the same Table 3 it could be notice that phosphorus had higher values in T_1 and T_2, it could be probably to the content of inorganic salt from a mixture of mono, di, tri, tetra, penta and hexa phosphates, while sodium content had been taken an opposite trend.

Health authorities recommend that sodium intake should not exceed 2000 mg day^{-1} that corresponds to 5 g of salt intake (WHO., 2011). The manufacturing process for processed cheeses, faces a more challenging situation as emulsifying salts, which contain sodium, are necessary to prevent the separation of fat and water after melting (Kapoor and Metzger, 2008). Thus, these cheeses contain another source of sodium in addition to the NaCl used as a raw material (Cruz *et al*., 2011). Only one the manufacture of low-sodium requeij cheese was found in the literature (Van Dender *et al*., 2010). Similarly, only one study describing a process for low-sodium Minas cheese (Gomes *et al.,* 2011).

The reducing of sodium content in cheeses is a multi-factorial problem that goes beyond the simple quantitative reduction of sodium in the food matrix. In particular, high levels of dietary sodium have been linked to hypertension and cardiovascular disease (He and MacGregor, 2007). Whilst, the proportions of Na in PCs depend on the types and levels of added ingredients, ES are generally a major contributor. Owing to their relatively high Na content, various approaches are used to reduce Na content of PCs, including reduction in ES content and the use of potassium phosphates/citrates, however, the latter approach is generally not favored because of the risk of predisposing consumers to hyper kalemia (Evans and Greenberg, 2005). On the other hand, inorganic phosphate in food additives is effectively absorbed and can measurably elevate the serum phosphate concentration in patients with advanced Chronic Kidney Disease (CKD). The main patho-physiological effect of phosphate is vascular damage, e.g., endothelial dysfunction and vascular calcification. Aside from the quality of phosphate in the diet, the quantity of phosphate consumed by patients with advanced renal failure should not exceed 1000 mg per day, according to the guidelines (Evans and Greenberg, 2005).

Nitrogen distribution of processed cheese: Type of emulsifying salt is closely affected the protein dispersion and nitrogen distribution. In the study of Fox *et al.* (2000), citrates were found to bind calcium weakly and show low peptization (the ability to form stable dispersions of colloidal particles in water),whereas other studies have shown that tri sodium citrate results is excellent calcium chelating ability and casein peptization properties (Cavalier-Salou and Cheftel, 1991; Mizuno and Lucey, 2005). Table 4 revealed nitrogen distribution in processed cheese samples. Protein solubility maybe attributed to the perturbation of the cross- linkages between the paracasein micelles, as confirmed by the removal of most of the calcium and inorganic phosphate from the paracasein.

Physical properties of processed cheese: Physical properties of processed cheese made with two types of ES were illustrated in Table 5. As shown in the Table 5, sodium citrate emulsifier salt had desirable parameters of penetration, melting index and oil separation. However, polyphosphate yielded substantially less meltability than citrate salt. This may indicated that the phosphate anion is a more functional Ca^{++} cross linker than the citrate anion in development of the internal structure of the protein matrix (Waugh, 1971). which could involve additional factors, such as molecular size, polarity, and strength of Ca^{++}bonds between anions.

It appears that Ca^{++}-cross-linking, which involves molecules composed of two or more condensed phosphate groups results in a spatial network with sufficient rigidity to resist collapse and flow when the temperature is elevated to near that of the melting point of milk fat. Blends of sodium-based emulsifier salts currently employed in cheese processing (Anonymous, 1976, 1977) achieve certain rheological and functional properties but also contribute to microbiological safety and stability against spoilage (Tanaka *et al*., 1979), especially at higher moisture contents and pH.

Color parameters: The type of emulsifying salts significantly ($p<0.05$) influenced all color parameters (Table 6). The cheese samples made with sodium citrate (T_1) were whiter and less yellow than the samples with phosphate salts (T_2). Generally, yellow color is associated with foods having a high fat content. However, the final color of the processed cheeses depends not only on the fat content, but also on the size of the fat particles dispersed in the protein matrix. According to McClements (2005), the smaller the fat particle diameter, the whiter the product.

Second part

Chromosomal Aberrations in bone marrow cells: The results of structural chromosomal aberrations are given in Table 7. Cytogenetic analysis in the present study showed the mean percentage of structural chromosomal aberrations and percentage of mitotic index of control and treated groups with T_1 and T_2. The results showed a high significant percentage in both total structure

Table 4: Nitrogen distribution of processed cheese samples prepared by two types of emulsifying salts

Values	T_1	T_2
TN (%)	2.250	2.266
SN (%)	0.3198	0.3449
SN/TN	14.220	15.216
NPN (%)	0.210	0.2431
NPN/TN	9.240	10.724
Relative casein	85.780	84.784

T_1: Made by organic salt (sodium citrate), T_2: Made by (commercial salt) polyphosphate

Table 5: Penetration, oil separation and melting index of processed cheese samples prepared by two types of emulsifying salts

Values	T_1	T_2
Penetration (mm)	182	155
Oil separation index (oiling off %)	20.00	25.33
Melting index (mm)	179	164

T_1: Made by sodium citrate, T_2: Made by commercial polyphosphate salt

Table 6: Color parameters of processed cheese samples prepared by two types of emulsifying salts

Values	T_1	T_2
L (black to white)	87.55	86.00
a (red to green)	-2.58	-2.01
b (yellow to blue)	25.99	24.88

T_1: Made by sodium citrate T_2: Made by commercial polyphosphate salt

Table 7: Mean percentage±SE of chromosomal aberrations in male mice bone marrow cells after treated with processed cheese

	Structural aberrations							
Treatments	Chromatid gaps	Chromosomal gaps	Chromatid breaks	Fragments	Deletions	Endomitosis	Total structural aberration	Mitotic index
Control	0.80±0.200	0.00±0.00	0.00±0.00	0.00±0.00	0.20±0.200	0.00±0.00	1.00±0.316	8.20±0.374
T_1	1.60±0.244	0.80±0.200	1.20±0.200	0.80±0.200	1.40±0.244	1.20±0.200	6.80±0.734	6.80±0.374
T_2	7.20±0.374	1.20±0.200	5.80±0.244	2.60±0.244	2.80±0.583	2.00±0.316	21.60±0.748	3.60±0.244

Table 8: Mean percentage of chromosomal aberrations in male mice after treated with processed cheese

	Structural aberrations											
	x-y univalent		Chain		Ring		Autosomal univalent		Total aberrations		Mitotic index	
Treatments	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	1	0.4	0	0.0	0	0.0	1	0.4	2	0.8	29	11.6
T_1	12	4.8	10	4.0	6	2.4	8	3.2	36	14.4	19	7.6
T_2	24	9.6	19	7.6	15	6.0	20	8.0	78	31.2	11	4.4

chromosomal aberration (gaps, breaks, deletion, fragments and endo-mitosis) and Mitotic index compared with control group.

Spermatocytes and mitotic index: The results of chromosomal abnormalities are given in Table 8. The results showed abnormalities in spermatocytes and mitotic index of mice administrated T_1 and T_2 for 30 days. It was noticed that all types of meiotic aberration (x-y univalent, autosomal univalent, chain, ring) were highly significantly as compared with control group. But mitotic index was significantly lower in treated groups than in control group.

DNA damage using comet assay: The results of the DNA damage are given in Table 9. It is clear that there was a significant increase in the tail length in blood cells of male mice treated with T_1 (10.40) and T_2 compared with the control mice group (0.80) and that clearly indicates a genotoxic effect of T_1 and T_2.

Micronucleus (MN) assay: The effect of T_1 and T_2 on micro-nucleated polychromatic erythrocytes (MNPE) in the bone marrow cells of male mice are given in Table 10. The present results in the control group of male mice showed a low amount of damaged cells. In contrast, the administration of T_1 and T_2 caused a highly significant increase in the frequencies of micronuclei in the cells of mice bone marrow compared with the control group.

Biochemical analysis

Percentage of malondialdehyde (MDA): The data in Table 11 represented the effect of T_1 and T_2 on serum malondialdehyde

Table 9: Visual score of DNA damage in male mice treated with processed cheese

		No. of cells		Class of comets					
Treatments	No. of animals	Analyzed	Total comets	0	1	2	3	DNA damaged cells (%)	DNA damaged (M±SE)
Control	5	100	4	96	3	1	0	4	0.80±0.200*
T_1	5	100	52	48	24	15	13	52	10.40±0.244**
T_2	5	100	89	11	32	29	28	89	17.80±0.374***

Class 0: No tail, 1: Tail length<diameter of nucleus, 2: Tail length between 1x and 2x the diameter of nucleus, 3: Tail length>2x the diameter of nucleus, *: No. of cells analyzed were hundred per an animal

Table 10: Micronucleated polychromatic erythrocytes (Mn PCEs) of male mice treated with processed cheese

	Mn PCEs/1000PCE	
Treatments and PCE screened	Number	Mean±SD
Control		
1000	6	6.40±1.140
1000	6	
1000	8	
1000	7	
1000	5	
T_1		
1000	16	16.20±1.483
1000	17	
1000	18	
1000	16	
1000	14	
T_2		
1000	29	29.80±0.836
1000	29	
1000	31	
1000	30	
1000	30	

Table 11: Percentage of malondialdehyde (MDA) in male mice treated with processed cheese

Treatments	Malondialdehyde (mmol g^{-1} tissue)
Control	0.60±0.244*
T_1	6.20±0.374**
T_2	15.60±0.509***

Data are presented as Mean±SEM

Table 12: Effect of treatment of processed cheese on serum biochemical analysis in male mice

	Liver function		Kidney function	
Treatments	ALT (IU mL^{-1})	AST (IU mL^{-1})	Creatinine (IU mL^{-1})	Uric acid (IU mL^{-1})
Control	28.70±0.76*	49.40±1.42*	0.45±0.01*	3.00±0.1*
T_1	40.73±1.37**	80.22±2.01**	0.73±0.02**	4.42±0.17**
T_2	54.25±1.61***	99.34±2.33***	1.87±0.11***	5.87±0.16***

Data are presented as Mean±SEM values marked with an asterisk, *: Significant different ($p< 0.05$), **: Significantly different ($p<0.01$) while, ***: Significantly different ($p<0.0001$)

(MDA) in liver tissues in oxidative stress model. The administration of male mice with T_1 (6.2%) and T_2 (15.60%) for 30 consecutive days caused a significant increase in the serum MDA and this increase were more frequent in T_1 and T_2 compared with the control group (0.60%).

Liver and kidney functions: The results in Table 12 represented the effect of T_1 and T_2 on serum ALT, AST and ALP (liver function) and creatinine level, urea and uric acid (kidney function) in male mice. The results of T_1 and T_2 showed highly significant increases in liver and kidney functions compared with the control group.

Up to date, no information has been published concerned the effect of cheese including tri- sodium citrate and sodium polyphosphate on genetic materials. However, several studies have been made on kidney function. The present study revealed that cheese containing sodium polyphosphate induced more toxicity in male mice than cheese containing tri- sodium citrate.

Phosphate salts refers to many different combinations of the chemical phosphate with salts and minerals. Foods high in phosphate include dairy products, whole grain cereals, nuts, and certain meats. Phosphates found in dairy products and meats seem to be more easily absorbed by the body than phosphates found in cereal grains. Cheese contains a lot of phosphate that they can cause too much phosphate in the blood. Several studies have shown that the association between high phosphate concentrations and higher mortality is not restricted only to persons with renal disease, it can also be observed in persons with cardiovascular disease and even in the general population. High normal serum phosphate concentrations are associated with coronary calcification in young, healthy men (Foley *et al*., 2009) and were found to be a predictor of cardiovascular events in the Framingham study (Dhingra *et al*., 2007). Elevated mortality in association with high-normal serum phosphate concentrations was seen mainly among persons with cardiovascular disease who had normal renal function (Tonelli *et al*., 2005).

In spite of we did not analyze the genotoxicity in heart tissues, we can suggest that increase phosphate concentration in male mice fed cheese with high concentration of sodium polyphosphate resulting in increase of genetic toxicity in heart tissues inducing cardiovascular disease.

It has been found that phosphate is resorbed in the intestines proportionally to the amount consumed in food, and that the resorbed phosphate was then excreted without any further difficulty by the kidneys. Normally, up to 80% of dietary phosphate is resorbed in the intestines, but the transport rate varies depending on the source of phosphate (and on the individual's vitamin D status). About two-thirds of dietary phosphate is eliminated in the urine, and on third in the feces (Berndt and Kumar, 2009). It has been discovered only in the last five years that phosphate homeostasis and the renal excretion of phosphate are regulated by a complex endocrine feedback system. The key hormone for phosphate homeostasis is Fibroblast growth factor 23 (FGF23) (Liu and Quarles, 2007). In genetically manipulated mice, the absence of FGF23 led to severe hyper phosphatemia and simultaneously to increase renal calcitriol synthesis by way of increased 1-alphahydroxylation (Shimada *et al*., 2004). On the other hand, an elevated concentration of FGF23 leads to increased renal excretion of phosphate and diminishes the activation of vitamin D to calcitriol.

It has been explanted that organic phosphate found mainly in protein-rich foods such as milk products are slowly hydrolyzed in the gastrointestinal tract and then slowly resorbed from the intestine. About 40 to 60% of the organic phosphate consumed in the diet is resorbed (Sullivan *et al*., 2009; Tonelli *et al.,* 2005). Therefore, the concentration of phosphate in the blood increases due to lack of absorption from the intestine which induce genetic toxicity and liver and kidney malfunction.

Compared to vegetable phosphate, it is found that the phosphates found in grains, nuts, and legumes are mainly in the form of phytic acid (hexa phosphoinositol), which cannot be split in the human intestine because of the lack of the enzyme phytase (Bohn *et al*., 2008). The bioavailability of vegetable phosphate is usually less than 50% (Tonelli *et al*., 2005; Lei and Porres, 2003) and thus much lower than that of the phosphate esters in protein-rich foods. It follows that the phosphate content of food cannot be automatically equated with the phosphate load.

CONCLUSION

In conclusion, fast food and ready-to-eat processed foods are the main contributors to today's rising dietary consumption of phosphate. Because of the increment of using food additives, the estimated daily intake of phosphate food additives has more than doubled since the 1990s, from just under 500 to 1000 mg day^{-1}. In processed cheese and other milk products, the phosphate content was nearly twice that of the natural product because of added phosphate. Thus, not only patients with impaired renal function need to be put on a low-phosphate diet; patients with cardiovascular diseases should also consume less phosphate, and indeed the general population should as well.

The previous study revealed that the type of emulsifying salts that used in processed cheese closely affected the properties of the final products and has a healthy risk that required further specific study.

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