

SOFT WHITE CHEESE AS A FUNCTIONAL FOOD ENRICHED WITH ANTIOXIDANTS NUTRIENTS MADE FROM ULTRAFILTERED BUFFALO'S SKIM MILK

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ABSTRACT

Manufacture white soft cheese has a potentially beneficial effect on health when consumer used, was the aim of this research. Some natural sources of antioxidant nutrients (phytochemicals) were evaluated its radical scavenging activity as antioxidant by using electron spin resonance (ESR). Green tea (extracted with different solutions; water, methyl alcohol and propylene glycol); garlic in two forms (oil and powder) and onion also in two forms (oil and powder) measurement by ESR. Results showed that water green tea extracted and onion powder have antioxidant efficiency 100%. UF- skim milk retentate, supplemented with water green tea extract (10 ml/Kg retentate) and onion powder (1g/Kg retentate) were used in processing soft white cheese. Modification in salt added through processing were carried out by reduced in sodium ions by using 2% (NaCl/KCl, 2:1) and fortify with zinc acetate (60 mg/Kg retentate). Two formula of soft cheese were produced; first supplemented with green tea extract and other supplemented with onion powder. Chemicals compositional characteristics and organoleptic examination were studied compared with control cheese. Results showed that little changes in chemical composition in cheeses, less in calcium activity in cheese supplemented with green tea related to chelating activity of tea components, and more increased in cheese made of onion may relate to the organosulpher components in onion. Organoleptic characters for cheese supplemented with antioxidant and less in sodium, more in zinc were less acceptable than the traditional cheese; but the biological value for its contents is more and that is proffered for healthy.

INTRODUCTION

There is no doubt that dairy products are functional foods. They are one of the best sources of calcium, an essential nutrient which can prevent osteoporosis and possibly colon cancer. Dairy products provide a spectrum of vitamins and minerals in addition to the basics of protein, fat, and carbohydrates. Other beneficial nutrients may be difficult to obtain from dairy sources alone, like antioxidants. The body can regenerate its own antioxidants, and we can get them from food. We are abundant in colorful fruits and vegetables. Many health professionals recommend adding an antioxidant supplement to our daily regime, especially if we're at risk for certain diseases.

Many common foods contain cancer-fighting from the antioxidants that neutralize the damage caused by free radical powerful phytochemicals (from plant sources). Other ingredients have enormous antiviral, anti-fungal, antibacterial parasitic, and heart health benefits. There are many compounds with so-called antioxidant properties that derived from natural sources.

Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. It is also increases in plasma antioxidant capacity in humans (Frei and Higdon, 2003).

Garlic is containing sulphur compounds has exerts antioxidant action by scavenging hydroxyl radicals (Prasad, *et al.* 1996; Yang *et al.*, 1993 and Kim *et al.*, 2001) superoxide anion (Kim *et al.*, 2001). Onions have a unique combination of three families of compounds that are believed to have salutary effects on human health- fructans (carbohydrate molecules that help maintain gastrointestinal health by sustaining beneficial bacteria); flavonoids (including quercetin and kaempferol) it functions as an antioxidant deactivating molecules that are injurious to cells in the body (Dorant *et al.*, 1994) and organosulfur compounds (such as cepaenes and thiosulfates that are largely responsible for the taste and smell of onions, Dorsch and Wagner, 1991; Goldman *et al.*, 1996).

Electron spin resonance (ESR) spectroscopy was used to evaluate the antioxidant capacity or its efficiencies as scavengers of free radicals. Antioxidant activity is determined by the inhibition percentage (%) of the intensity of the superoxide or hydroxyl dependent ESR signal. These antioxidants were investigated for radical scavenging activities using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), (Chen, *et al.*, 2002; Hou, *et al.*, 2004). The aim of this research is manufacture functional soft cheese from pasteurized ultrafiltered skim milk supplemented with different natural sources of antioxidants and get product higher in protein with reducing in lactose content; and increasing in biological value.

Calcium chloride is often added to milk soft cheese manufacture. Information concerning the effects of CaCl_2 on coagulation should help understand the mechanism of acidic coagulation of casein and allow better control of curd texture (Harwalkar and Kalab, 1981). Jablonka and Munro (1986) suggested that Ca^{+2} from crosslinks between negatively charged groups of the caseins, resulting in tighter, more compact curds and larger curd particles.

Sodium chloride is widely used as preservative and flavouring agent in cheese manufacture. High sodium intake has been claimed to be a major contributor to development of hypertension and cardiovascular diseases (Albernetly, 1979 and Anonymous, 1980). Studies showed that partial replacement of sodium chloride by potassium chloride in manufacture of domiati cheese using mixture of NaCl/KCl at the rate of 3:1 and 2:1 gave good quality cheese (Ramadan, F.A.M., 1995), (Salem and Abeid, 1997).

Although trace minerals are not true antioxidants, minerals have an important role in the effective functioning of antioxidant enzymes. Zinc is very essential for metallothionein leading to suppressing of free radicals. Diet can be fortified with zinc salt like zinc acetate. Zinc is one of the most important elements for body metabolism. It participated in the mechanisms of the major metabolic pathways involving proteins, carbohydrates, lipids, and nucleic acids synthesis. As zinc having this key role for metabolism in the body, its deficiency will affect markedly this role (Fayad and Abou Zikri, 1997). Milk is considered to be deficient in zinc (Jarrestt, 1979), even it is one

of trace elements in milk (Jayasekare, *et al.*, 1992). Several investigators have been done for enriching milk and some dairy products with zinc such as for drinking purposes: (Hermanson *et al.*, 1995; Steils *et al.*, 1994); for cultured milk (El-Loly and Hofi, 1999); Yogurt (Degheidi, 1998; Abd-Rabou *et al.*, 1999); Manchego-type cheese (Spain) (Moreno *et al.*, 1994) and Domiati cheese; (Hagrass *et al.*, 1984; Darwish *et al.*, 1989 and Abd-Rabou, N.S.2002). Zinc acetate was used to fortify cheese. Zinc acetate selected due to bioavailability of zinc in humans, according to Bobiya, *et al.* (1991) and Castillo & solomons (1991).

MATERIALS AND METHODS

MATERIALS

Ultrafiltration process (Preparation of retentate).

Buffalo's milk retentate was prepared in Animal Production Research Institute, Agric.Res.Center, El Dokki; Giza. The pasteurized skim milk containing 0.7 to 1% fat was cooled to 50-55°C before ultrafiltration, which carried out to concentration factor 4.

Starter culture:

A mixture of yogurt culture consisting of 1 ml each of *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*, (1:1) from MERCEN- Agriculture College; Ain Shams Univ.

Salts used in cheese manufacturing

Calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$), zinc acetate was used to fortify at level of 60 mg Zn/Kg milk (Abd Rabou, 2002); and mixture of sodium chloride and potassium chloride (NaCl/KCl at the rate of 2:1). from MERCEN- Agriculture College; Ain Shams Univ.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (10^{-2} M)/ 50 ml methanol. from MP Biomedicals, LLC, Germany.

Commercial rennet

DSM, Fromase® 2200TL, Granulae, France rennet was used. The rennet concentration used was based on the normal amount used in Domiati cheese making, i.e., 0.04g per Kg milk.

Antioxidant nutrients

Chinese green tea with different extract solution:

Preparation of green tea extracted:

Chinese green tea was purchased from a supermarket. Different extract solutions were used to recognize if there are different in antioxidant potential depend on the type of extract. Types solutions used to dissolve tea were distilled water, ethanol and propylene glycol. Five grams of tea was infused into 100 ml of solution, and then filtered through Whatman No.4 paper to get extracts.

Garlic (oil and powder) and Onion (oil and powder).

Garlic purchased from El-Basha comp. Cairo, which export this oil to Europe. Oil was diluted with an oil-soluble liquid (olive oil) (mix 100 µL essential oil in ~ 500 µl of olive oil). Powder was dissolved in distilled water (1g in 100 ml D.W).

METHODS

Evaluation the antioxidant activity by scavenging activities of DPPH (stable radicals) using electron spin resonance (ESR) spectrometry.

Antioxidant activity of antioxidant solutions were evaluated by electron spin resonance spectrometry (ESR) (BRUKER) to evaluate their radical scavenging properties against stable DPPH radical. DPPH is considering the source of the hydroxyl radicals, which mixed with antioxidants sources, to evaluate the antioxidant activity against free radicals. The mixture solution (500 µL) was transferred to an ESR silica tube and placed at the cavity of the ESR spectrometer. Deionized water was used instead of antioxidant solution for blank experiments. After 40 s, the relative intensity of the signal of the DPPH-OH spin adducts was measured by the procedure of (Hou, *et al.* 2004). All ESR spectra were recorded at the ambient temperature. The conditions of ESR spectrometry were as follows: center field, 345.4 (5.0 mT; microwave power, 0.00202148 [W]; microwave frequency [9.773 Hz]; modulation amplitude, 5 G; modulation frequency, 100 KHz; time constant, 0.5 s; scan time, 1.5 min. ESR spectra were recorded on a Beucker, ELEXSYS E-500, ESR spectrometer operating in X-band frequency. The relative signal intensity was calculated using a manganese internal standard by measuring the peak-height of the second line in the spectrum. Products with no antioxidant activity cause the ESR signal to remain at the level as the standard. The radical scavenging ability of natural sources of antioxidants was calculated as the degree of inhibition (I_{ESR}), or

$$\text{Antioxidant efficiency \%} = \frac{\text{DPPH}_{\text{area under peak}} - \text{antioxidant sample}_{\text{area under peak}}}{\text{DPPH}_{\text{area under peak}}} \times 100$$

DPPH_{area under peak}

Cheese manufacturing

UF-retentate at 42°C, addition these salts, Calcium chloride (0.02 % w/w), mixture of NaCl/KCl at the rate of 2:1 (2% w/w) and zinc acetate (50 mg /kg retentate) were added to UF-retentate at 42°C and thoroughly mixed. Water tea extract with (10 ml/ Kg retentate) and onion powder (1g / kg retentate) were added to the retentate into different treatments. The retentate was inoculated with well stirred yogurt starter 1% and left 15-20 min, and 1.5 ml of standard rennet solution / Kg retentate was added. The mixture was stirred and dispensed into 150 ml plastic containers and left 20-30 min at 40°C until coagulation carried out. Containers kept in refrigerator (7°C). Cheese samples were taken for analyses after one day. Control cheese included (2% NaCl), without zinc acetate or antioxidants sources.

Compositional of fresh soft white cheese analysis:

Cheese samples were analyzed for total solids, fat, ash content as described by Ling (1963). Total nitrogen was determined by micro-Kjeldahl

method ; a factor of 6.38 was used to calculate protein and titratable acidity were determined according to (AOAC, 1980) and pH was measured by an Orion pH meter. Lactose was determined according to the method described by Nickerson *et al.* (1975). Calcium activity in permeate drained from cheese was determined according to Nieuwenhuijse, *et al.* (1988). Zinc concentration in functional cheese was determined according to AOAC (1990).

Organoleptic properties

Cheese samples were scored for flavour (50 points), body and texture (35 points); appearance and colour (15 points). The score was averaged by regular score panels of the staff members of the dairy department, according to Nelson and Trout (1981) and Hassan *et al.* (1983).

RESULTS AND DISCUSSION

Evaluation the antioxidant activity by scavenging activities by electron spin resonance (ESR) spectrometry.

Rate of DPPH radical disappearance in the presence of antioxidants solutions are measured by ESR spectrometer and is also used to assess free radical scavenging ability of antioxidants solutions

DPPH is a stable free radical capable to accept electron from reactive radicals, thus behaving as a radical scavenger ((Yordanov, 1996). Additionally, DPPH acts as an electron acceptor from antioxidants (HA) and several electron transfer reactions of DPPH with phenols, amins and other compounds were described in literature (Stasko, *et al.* 2002; Yordanov, 1996).

Figure 1.a shows ESR signals for DPPH free radicals. Figure 2. shows ESR signals for DPPH and green tea extracts mixtures. Figure (2.a) for DPPH with green tea extracted by distilled water, Fig.2.b. for DPPH with green tea extracted by methyl alcohol and Fig.2c. belong to DPPH with green tea extracted by propylene glycol. Figure 3a shows DPPH with garlic oil and Fig.3b belong to DPPH with garlic powder. Figure 4 a shows DPPH with onion oil and Fig.4b shows DPPH with onion powder.

The radical scavenging abilities of antioxidant solutions were calculated the double integration area; as the degree of quenching of the radical anion (I_{ESR}) were found that the radical scavenging ability of green tea extract with all solutions (0.5 μ M; I_{ESR} = 100 %), garlic powder (0.5 μ M; I_{ESR} = 45%) was higher than garlic oil = (0.5 μ M; I_{ESR} = 38.6 %) whereas onion powder (0.5 μ M; I_{ESR} = 100 %) was higher than onion oil = (0.5 μ M; I_{ESR} = 27.8 %). It was unacceptable that onion powder has antioxidant efficiency equal 100%. In same time, green tea with different solutions for extract gave the same efficiency as antioxidant. Onion powder and green tea extracted by water were used as source of antioxidants in processing soft cheese by UF.

Chemical composition of soft cheese:

Table(1): Chemical composition of fresh soft white cheese made by Ultrafiltration method.

Contents %	Control cheese	Cheese with Tea	Cheese with onion
Total solids	28	28.5	29
Fat	2.5	2.5	2.7
Total protein	13.60	13.2	13.7
Lactose	4.9	4.7	4.8
Salts	3.45	3.5	3.6
Ca ²⁺ (mg/100g)	0.87	0.77	0.82
Zinc (mg/L)	11	65	67
Ash	3.4	3.51	3.64
pH	6.7	6.24	6.43
Titrateable acidity	0.16	0.28	0.25

Means of duplicate analyses.

Sensory evaluation of soft cheese:

Table 2: Data illustrated in Table 2 showed the organoleptic total score of control cheese, cheese supplemented with green tea extract and cheese with additional onion powder.

Type of cheese	Organoleptic scores			Total scores (100)
	Flavor (50)	Body & Texture (35)	Appearance & color (15)	
Control cheese	46	33	15	94
Cheese with tea	43	30	11	84
Cheese with onion powder	40	28	12	79

DISCUSSIONS

Natural antioxidant sources that appeared high activity and used in cheese processing were water green tea extract and onion powder. Antioxidant activity of onion is related to Quercetin and kaempferol (the major flavonoids in onions) are the flavonol subclass. Quercetin provide for free radical scavenging is presence of *ortho*-dihydroxyl (catechol) structure in B ring, 2,3-double bond in conjunction with 4-oxo function in C ring and additional presence of 3- and 5-hydroxyl groups (Yokozawa *et al.*, 1999; Kaneko and Baba, 1999). In green tea extract, The major source of phenolic compounds in green tea extract include the flavanols epicatechin, epigallocatechin, and their gallate esters which can scavenge reactive oxygen radicals, such as the hydroxyl radical and superoxide anion radical, by donating a hydrogen atom or electron (Rice-Evans *et al.*, 1996 and 1997).

Cheese made with green tea extract showed more rigidity and less in Ca²⁺ and more acidity; because polyphenols (catechin and epicatechin, the major polyphenolic constituents of green tea) have ability to interact with protein. Hydrophobic and hydrogen bonding are responsible for polyphenol-protein interactions (Spencer *et al.*, 1988; Haslam and Lilley, 1988), whereas, caffeic acid help to lower pH, besides its ability to chelate calcium (O'Connell and Fox, 1999). At lower pH, micellar calcium phosphate (MCP) will

dissolved (Degleish and Low, 1989) and casein (in the enzymatic stage of the renneting reaction) has negative charged related to negatively charged of phosphoserine residues and glutamic acid ($pK \approx 4.6$) and the binding of H^+ ions to phosphoserine and glutamic acid increases. By adding Ca^{2+} , calcium ion activity will increased, leading to more shielding of negatively charged groups of the casein and forming crosslinks between negatively charged groups of casein, with more interaction within the gel network (Jablonka and Munro, 1986).

Cheese made with onion powder showed little softy than control. Onion is rich in organosulphur compounds; that with decreasing in pH that encourage the formation of protein-k-casein protein from the casein micelles, before Ca^{2+} ions play its role in building the network structure.

Chemical analysis showed that nutrients supplemented partially interact with milk components during cheese manufacture and its contents percents. This may related to a buffering effect of milk that protects chemical degradation of the supplemented during the processing.

CONCLUSIONS

ESR technique showed that green tea extract with water gave the same activity as antioxidant like the extract with other solvent that is undesirable used in diet. Onion powder also gave high efficiency as antioxidant and it is easier used in supplementation diet than oils. In same time these components appeared easily mixer in milk and did not appear any problem during cheese processing.

Chemical composition did not affect significantly by this supplementation; whereas organoleptic tests may little effected comparing to the control. Biological effect of this healthy product will make the consumer be happy compared with taste or color. This cheese has low fat content, which became preferred by many people for perceived health reasons. Zinc mineral was also supplemented, because it is vital for immunity, growth and development. Reduced sodium ions in cheese was also taken in consider to get functional dairy product (healthy cheese) needed by many peoples. This product is consider functionally food, that included many characteristic, low - fat dairy product; including functional ingredients "antioxidant nutrients" phytochemicals and fortify with zinc. Antioxidant activity can be used on the dairy product label

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انتاج جبن أبيض بطريقة الترشيح الفائق من لبن فرز جاموسي مدعم بمضادات الأكسدة كغذاء وظيفي

أحمد سعد جاد - هاله محمد فخر الدين

المركز القومي للبحوث - قسم الألبان

استخدم جهاز Electron spin resonance في تقدير كفاءة مضادات الأكسدة لكل من مستخلص الشاي الأخضر، الثوم، البصل. رنقد تم استخلاص الشاي الأخضر بالثلاث طرق التالية : بالماء المقطر الساخن ، مذيب الميثانول ، البروبيلين جليكول (٥ جرام شاي جاف / ١٠٠مل مذيب) أما الثوم والبصل فكان على صورتين زيت وبودرة وتم اذابة البودرة في ماء مقطر (اجرام بودرة / ١٠٠ ماء مقطر)

وبتقدير كفاءة المستخلصات كمضادات للأكسدة بجهاز ESR لهذه المصادر فقد أعطى مستخلص الشاي بكل طرق الأستخلاص أعلى كفاءة (١٠٠%) كذلك بودرة البصل قد أعطت أيضا أعلى كفاءة وكانت (١٠٠%) استخدمت المصادر ذات الكفاءة العالية كمضادات الأكسدة في تدعيم الجبن الأبيض المصنع بطريقة الترشيح الفائق واستخدم اللبن الفرز الجاموسي للحصول على منتج منخفض الدهن اما ملح الصوديوم المستخدم في التصنيع فقد أختزل عن طريق استبدال جزء منه ملح البوتاسيوم وكانت النسبة الكلية المستخدمة ٢% كذلك تم تدعيم المنتج بأسيئات الزنك ٦٠ملجم / كجم مركز أوضحت النتائج أن التركيب الكيميائي لكل من الجبن المدعم بمستخلص الشاي وبودرة البصل لم تختلف بصورة واضحة إلا أن نسبة الكالسيوم الحر في الراشح الناتج من الجبن المصنع بالشاي الأخضر كان أقل من الجبن المصنع بدونه كذلك يقل قليلا من الجبن المصنع ببودرة البصل كذلك ارتفاع نسبة الدهن بنسبة قليلة في الجبن المصنع ببودرة البصل كما أوضحت الأختيارات الحسية حصول الجبن المضاف لة بودرة بصل اعطى نقاط أقل من عينة المقارنة أما الجبن المصنع بالشاي الأخضر فكانت نقاطه اعلى من الجبن المصنع ببودرم البصل إلا أن ذلك تم تعويضة لإرتفاع القيمة الحيوية لمنتج الجبن المدعم لما تحتويه على مضادات الأكسدة وانخفاض أيون الصوديوم ودعمه بأيون الزنك لذلك يمكن أن يطلق عليه غذاء وظيفي