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RESEARCH ARTICLE

Using of Whey Proteins as Antioxidant in Some Food Products

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Abstract

Oxidation of foodstuffs has become an issue concerning with food quality and consumer health, specifically in dairy products which contain a percentage of fat. The present study aimed to use whey proteins concentration (WPC) as additive to some samples of dairy products selected from local market. Adding WPC to these products with different concentration demonstrated a good activity of whey proteins concentration as antioxidant. Lipid oxidation measured on the basis of malondialdehyde by determining thiobarbaturic acid (TBA). Results obtained show differences in TBA value before and after adding WPC. Adding WPC reduced the TBA value about half in collected samples, and that led us that the period of lipid oxidation with adding WPC will be longer than these samples used without adding WPC. Using this method to protect food products from lipid oxidation by adding WPC has been proved the activity of whey proteins as antioxidant in this research.

Keywords: *Whey proteins, Antioxidant activity, Foods products.*

Introduction

The significance of oxidation forms in human body and food products became an important issue. In food products, oxidative responses cause deterioration in food quality, inadmissible taste or texture, color, decrease in nutritive value at whole and reducing the validity of food products. Compounds which are in charge of ruinous and deadly cell impacts are lipid peroxides and low atomic weight compounds produced during late phase of the oxidative responses.

The principle focuses of these reactions are lipids, proteins, DNA and compounds. For protecting food products from deterioration and for giving protection against the advancement of age-particular illnesses like cancer, atherosclerosis and diabetes, it is important to restrain lipid oxidation occurred in food products and in human body [1].

The stability of poly unsaturated fatty acids in food is a frequent problem, because these fatty acids cannot stand against lipid oxidation, and that leads to damage these unsaturated fatty acids and thereby a decrease in their nutritional value [2].Therefore, effective strategies are required for protecting poly unsaturated fatty acids in foods from oxidative crumbling. Increasing attention toward health, consumer protection and the concerns regarding the use of synthetic anti-oxidants as well as consumer's choice of food products offered in the market led to the needs for recognition and using of natural antioxidants that can protect human from many chronic diseases [3].

At present time, the dairy industry in many countries has been developed new techniques through the addition of non-structural materials such as plant proteins, fresh vegetables and fruits [4], herbs and other plants that have benefits to the health, such as antioxidative in thyme (*Thymus vulgaris*) and Beetroot [5, 6].In recent years, scientific evidence demonstrated that bioactive peptides and proteins in foods could have several advantages effects on human health [7]."Bioactive peptides, which are inactive within the sequence of their parent proteins, can release by enzymatic hydrolysis during

gastrointestinal transit, fermentation (e.g., milk), ripening (e.g., cheese), and food processing. Biologically active peptides are defined as protein fragments that remain inactive in their precursor sequences, but when released by hydrolysis with proteolytic enzymes, may interact with selected receptors and control physiological functions. Bioactive peptides can be obtained by proteolytic microorganisms or hydrolysis by digestive enzymes or microbial proteases"[8].

Antioxidant peptides usually consist of 5-11 amino acids, including proline, histidine, tyrosine, tryptophan and hydrophobic amino acids. The antioxidant action is concerning with the exposition of ionizable and hydrophobic groups" [9]."Whey proteins, a by-product recognized as valuable food ingredient with important nutritional and functional properties is getting acceptance as functional food ingredient.

Hydrolysis of whey proteins is known to release bioactive peptides that can exhibit a number of physiological properties, such as immunomodulatory, anticancer, opioid, hypocholesterolemic, angiotensin I-converting enzyme (ACE)-inhibitory, antidiabetic, antioxidant, chelating and antimicrobial activities. Also, the antigenic determinants present in α-lactoalbumin (α-LA) and ß-lactoglobulin (ß-LB) are eliminated through hydrolysis. Therefore, whey proteins hydrolysates are promising from nutritional, therapeutic and technologic perspectives, in the form of crude extracts or semi-purified peptide extracts"[10].

Whey proteins have high solubility over a wide pH range. With low level of fat and lactose in these products make them perfect ingredient for formulating sugar free, low fat or no fat dairy products"[11]. "Whey proteins contain high levels of branched-chain amino acids (BCAAs), i.e., leucine, isoleucine and valine.

Leucine is an important factor for tissue growth and repair and has been recognized as a key amino acid for the initiation of translation. Whey proteins are also rich in the sulphur containing amino acids cysteine and methionine. These amino acids enhance immune function upon intracellular transformation to glutathione, an effective antioxidant"[12].The objective of this research was to prove the antioxidant activity of whey proteins concentrations (WPC) as antioxidant, hence the present research was designed to evaluate the impact of enrichment of some dairy products with WPC. WPC has improved their antioxidant activity in collected samples.

Materials & Methods

The sweet whey was obtained from the Food Science Department/Teaching Plant in the Faculty of Agriculture/University of Baghdad. One Liter sample of whey were filtered to remove fat and casein particles by vacuum filtration using Whatman filter paper number 40.Whey was heated for 15 min. at boiling point to coagulate whey proteins.

The protein free whey was filtered by Buchner funnel through Whatman filter paper No. 1 and the supernatant was collected and wash with water to get rid of salts and lactose sugar until the washing water became clear . The proteins were dried in the oven at 45 C° and the dried was tried by Moulinex coffee grinder and stored at -20°C in airtight container.

WPC Composition

Whey proteins concentration analyzed to determine their composition of: Moisture, Ash, protein, fat and lactose. WPC were determined in accordance with [13]. Analysis realized at Central Laboratory of Agricultural Engineering Science/University of Baghdad. Results of WPC composition indicated in Table 1.

Preparation of Hydrolysis Whey Proteins

The acid used to hydrolysis of WPC to amino acid composition include, a 2% (wt/vol) WPC solution was hydrolyzed with (0.03 N HCl, pH 2.5). After hydrolysis, the solution were heated at 100°C for 10 min and centrifuged at 2,000 × g for 20 min to remove undigested proteins. The resulting supernatant was dried and used as WPC hydrolyses.

Collection of Samples

Ten samples of various dairy products with different fat percentage divided into (4 samples cream milk 30% fat, 3 samples cream cheese 40% fat and 3 samples soft cheese 20% fat) were collected from local market in Baghdad city for testing in the

chemical laboratory of Market Research and Consumer Protection Center / University of Baghdad.

Adding WPC as Antioxidant

Different amount of WPC (0.1, 0.2, 0.3, 0.4, and 0.5 gram/100 gram) were added to the collected samples, to show the different in TBA value. TBA value measured after storage to 3, 6, 9, 12 and 15 days.

The thiobarbituric Acid Assay (TBARS)

The lipid peroxidation realized on the basis of malondialdehyde (MDA) levels, it was carried out using the method described by (Witte et al.1970) [14]. Briefly, 1 g of WPC and 25 ml solution of (20%Trichloro acetic acid (TCA) dissolved in phosphoric acid 2M) were mixed in Naturalization device for 2 min, the mixture was transferred to a volumetric flask 50 ml and the volume was brought up to 50 ml with distilled water. 25 ml of the mixture was centrifuged for 30 min at 3000 rpm; the mixture was filtered through filter paper no. 1.

Five ml of filter was mixed with 5 ml of TBA (0.005M) dissolved in distilled water. Five ml of distilled water mixed with 5 ml of TBA as blank. The test tubes were mixed and allowed to stand for 15-16 h at room temperature. The absorbance was measured at 530 nm. The lipid peroxidation products in the tested samples WPC were estimated by the formation of thiobarbituric acid reactive substances (TBARS) and quantified in term of malonyldialdehyde (MDA). Reducing of TBARS value formation after adding WPC was calculated compared with control. TBARS value was calculated According to equation: TBARS value (mg/ kg) = A_{530} **× 5.2**

Results & Discussion

Results of analysis of whey proteins concentration (WPC) composition is shown in Table (1). The pH of the sample was 5.42. The percentages of moisture, ash, fat, protein and lactose were 0.148, 0.058, 8.12, 38.18 and 5.3% respectively. WPC considered as antioxidant in this research. Collected samples prepares to measure lipid peroxidation on the basis of malondialdehyde by determine thiobarbaturic acid (TBA) as the method mentioned above. UV-1100 Spectrophotometer used to measure absorbency of prepared samples before and after adding 0.1, 0.2, 0.3, 0.4 and 0.5 gram of whey proteins concentration (WPC).

TBA value calculated by using equation: TBA mg/kg=Absorption (530) ×5.2 Results obtained show differences in TBA value before and after adding WPC. Adding WPC reduced the TBA value about half in collected samples and that led that the period of lipid oxidation with WPC adding will be longer than these samples used without WPC. TBA value in collected samples were reduced to the half after adding WPC and this conduct us that WPC can used as antioxidant in collected samples of dairy products in this research. Results of TBA value of all collected samples are illustrated in Table 2, 3 and 4.

Table 1: Chemical composition of whey proteins concentration (WPC)

	pH	Moisture	Ash	Fat	Protein	Lactose
WPC	5.42	0.148%	0.058%	8.12%	38.18%	5.3%

In Table (2) shows the antioxidant activity of WPC in cream milk samples. From the results it has been demonstrated that adding WPC to cream milk samples 1 to 5 in concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 gram/100 gram respectively, has been reduced TBA value in the samples from 0.275, 0.228, 0.197, 0.171 and 0.145 mg/kg respectively. It seems that adding WPC functions as antioxidant and protects samples from deterioration and lipid oxidation longer. It was revealed that the antioxidant activity of whey proteins not only depend on their protein quantity, but also on their protein composition [15] "Antioxidant activity of whey proteins is due to the chelation of transition metals by lactoferrin and scavenging of free radicals by sulphur containing amino acids". [16]. "WPC which are rich in sulfur amino acids, cysteine and methionine that can reduce common lipid oxidation" [17].

Table 2: Antioxidant activity of (WPC) in cream milk

Samples	Days	WPC (g.) adding	Absorption (530)	TBA value mg/kg
Control	0		0.060	0.312
Cream Milk 1	3	0.1	0.053	0.275

Cream Milk 2	6	0.2	0.044	0.228
Cream Milk 3	9	0.3	0.038	0.197
Cream Milk 4	12	0.4	0.033	0.171
Cream Milk 5	15	0.5	0.028	0.145

The TBA value of cream cheese samples were shown in Table 3. From the results it has been demonstrated that adding WPC to cream cheese samples 1 to 5 in concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 gram/100 gram respectively, has been reduced TBA value in the samples from 0.582, 0.509, 0.473, 0.452 and 0.421 mg/kg respectively. That proved the antioxidant activity of WPC has been reduced the rate of oxidation upon adding to cream cheese samples and improving the shelf- life of cream cheese samples.

Table 3: TBA value for cream cheese

Samples	Days	WPC (g.) adding	Absorption (530)	TBA Value mg/kg
Control	0		0.118	0.613
Cream Cheese 1	3	0.1	0.112	0.582
Cream Cheese 2	6	0.2	0.098	0.509
Cream Cheese 3	9	0.3	0.091	0.473
Cream Cheese 4	12	0.4	0.087	0.452
Cream Cheese 5	15	0.5	0.081	0.421

In the Table 4 showed the TBA value for soft cheese. From the results it has been demonstrated that adding WPC to soft cheese samples 1 to 5 in concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 gram/100 gram respectively, has been reduced TBA value in the samples from 0.785, 0.722, 0.634, 0.572 and 0.494 mg/kg respectively. Adding WPC to soft cheese samples has been considered as antioxidant and protects samples from oxidation long time than same samples without adding WPC.

Table 4: TBA value for soft cheese

Samples	Days	WPC (g.) adding	Absorption (530)	TBA Value mg/kg
Control	0		0.165	0.858
Soft Cheese 1	3	0.1	0.151	0.785
Soft Cheese 2	6	0.2	0.139	0.722
Soft Cheese 3	9	0.3	0.122	0.634
Soft Cheese 4	12	0.4	0.110	0.572
Soft Cheese 5	15	0.5	0.095	0.494

"Malondialdehyde (MDA) relased by the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbaturic acid (TBA), produced a pink-red chromogen with an absorbance measured at maximum 530. In the present study, TBARS was used as indicator of lipid peroxidation which is a useful test for the measurement of antioxidant activity of several compounds [18].And to protect unsaturated fatty acids from oxidation. Using this method to protect food products from lipid oxidation by adding WPC has been proved the activity of whey proteins concentrations as antioxidant in this research.

Conclusion

"The antioxidant capacity of whey proteins has been attributed to the presence of hydrophobic amino acids such as tyrosine, methionine, histidine, lysine and tryptophan "[19].Results demonstrated that the antioxidant activity in samples after 15 days is higher than Control. WPC succeeded to reduce the lipid oxidation until 15 days and considered as antioxidant.

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