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ANTIOXIDANT PROPERTIES OF PROCESS CHEESE SPREAD FORTIFIED WITH ACID CASEIN HYDROLYSATE

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Abstract

Acid casein hydrolysates were prepared by enzymatic hydrolysis using pepsin for 40 min with constant agitation. The acid casein hydrolysate was added at different levels (0, 10, 20, 30, 40 and 50%) into process cheese spread blends made from young Cheddar cheese (15 days storage) just before processing. The chemical composition, radical scavenging activity, total free amino groups and sensory properties of the products were evaluated. The results showed that the radical scavenging activity of the resultant cheese increased with increasing the concentration of casein hydrolysate. Also, free amino groups were estimated in process cheese while fresh and during storage (20,40 and 60 days) at 7°C ± 1°C, the results were represented as the increase in optical densities (O. D). The obtained O. D. values increase with increase the concentration of casein hydrolysate. The O.D values increased to 0.976, 0.986, 1.023, 1.137, 1.164 and 1.203 after 60-day storage in the same order. Moreover, cheese preparations underwent sensory evaluation for appearance, aroma**,** taste, mouth feel, color, overall quality and total score. In general, no significant differences (P <0.05) were observed between control sample and treated samples with 10, 20 and 30% casein hydrolysate for total scores therefor, these samples can be grouped together. However, the cheese made with 40 and 50% casein hydrolysate received significantly (P < 0.05) lower scores and water phase separation was observed after 60 days storage with 50% treatment.

Introduction

In humans, accumulation of free radicals causes many diseases such as cancer, autoimmune

disorders, aging, diabetes, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Naskar *et al.,* 2011 and Islam *et al.,* 2013). Antioxidants can scavenge free radicals and diminish their effect. Therefore, the search for naturally occurring antioxidants of animal and plant origins are imperative. A free radical is unstable atom or molecule therefore, it has the tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells (Gilgun-Sherki *et al.,* 2002; Choe, & Min, 2009). Antioxidants are substances can prevent the destructive effect of free radicals whether directly by pairing their electrons or indirectly by reaction with the unstable intermediate compounds. The biological systems of human beings have their own antioxidant defense that able to remove damaged molecules, but these native antioxidant defense can be inefficient. Therefore, consumption of antioxidants-rich food is valuable to protect cells from damage caused by free radicals. Antioxidants from natural sources are more superior to those produced chemically, because some synthetic antioxidants have been reported to be carcinogenic (Liu *et al.,* 2005). A number of bioactive peptides have been identified in milk protein hydrolysates and fermented dairy products (Al-Saleh et al 2014; Miao et al 2019). Cosentino *et al.,* (2010) reported that upon hydrolysis with certain enzymes or acids, milk proteins can act as antioxidants in model systems. The ability of peptides to inhibit lipid oxidation appears to be related to certain amino acid residues in the peptides, such as tyrosine, methionine, histidine, lysine, and tryptophan, which are capable of chelating prooxidative metal ions (Diaz *et al.,* 2003; Choe and Min, 2006). Milk caseins may inhibit lipid peroxidation possibly through the mechanism of increasing iron autoxidation (Mansi et al 2020). Peptides from casein hydrolysate have been shown to possess superoxide anion scavenging activity (Liao *et al.,* 2019; Liu *et al.,* 2020) reported that tryptic casein hydrolysate with MW = 779.4960 Da has a strong calcium binding activity (129.46 mg g), enhancing calcium transport and absorption causing bone integrity. Furthermore, Horner *et al.,* (2019) stated that a significant reduction in blood glucose was found in participants consumed 12 g casein hydrolysate compared to the intact casein (sodium caseinate). To our knowledge, there are no studies about the effect of acid casein hydrolysate addition on the properties of process cheese spread.

So, this study was aimed to incorporate acid casein hydrolysate into process cheese formula and to evaluate the antioxidant and free radical scavenging activity as well as sensory evaluation of the resultant cheese.

MATERIALS AND METHODS

1- Materials and Chemicals

Diphenyl-2-picrylhydrazyl (DPPH), Trypsin, pepsin sodium hydroxide and O-phthaldialdehyde (OPA) were purchased from Sigma

Chemical Co. (St. Louis, MO, USA); potassium citrate, citric acid, sodium mono-phosphate, sodium diphosphate (phosphate buffer) were purchased from Sigma-Aldrich. Emulsifier salts (JOHA S9 and JOHA S85) were obtained from JOHA BK Ladenburg crop., GmbH, Ladenburg, Germany Purchased from local market. The other chemicals were of analytical grade. Butter oil 99.8% milk fat (fern) was purchased from IFFCO group Co. New-Zealand, Regilait skim milk powder obtained from REGILAIT- 7118 Saint Martin, belle roche, Franc, Rennet was obtained from the Dairy Sci., Dept., Fac., of Agric., Minia Univ. *Lactococcus lactis* ssp *lactis and Lactococcus lactis* ssp *cremoris* were obtained from ATCC (American Type Culture Collection, Rockville, Md., USA).

2-Methods

The moisture was determined according to the method as described by Bradley and Vanderwarn, (2001); AOAC, (2007) respectively. Fat and protein contents were analyzed using Gerber method (Van Gulik (1975) and the Kjeldahl method (AOAC, 2007), respectively. The pH was measured using a digital pH meter (pH 211, Hanna instruments microprocessor pH meter, (model SA 720, USA).

2-1-Cheddar cheese manufacture

The manufacturing of Cheddar cheese was modified from Walstra *et al.,* (2006).

2-2- Acid casein preparation

Acid casein was prepared from raw cow skim milk according to **Mulvihill and Ennis, (2003).**

2-3- Process cheese preparation

In the current study, six process cheese spread formulations were prepared using natural 15 days age Cheddar cheeses with chemical composition indicated in Table (1) and acid casein hydrolysate. Each formulation was designed to achieve end-products with 55% moisture, 25% fat, 14% protein, and 3% emulsifier salts (to meet the Food Drugs Administration (FDA); CFR 2014 requirements)

Table 1. Composition of Cheddar cheese used in the manufacture of process cheese spread.

composition	Percentage
Moisture	35
Protein	25
Fat	35
Ash	4.1

The detailed ingredient blends and formulations of the six-process cheese spread are shown in Table 2. The amount of cheddar cheese was calculated in control sample to be 416.67 g/kg process cheese (≥ 51% of the dry matter of the final process cheese product, CFR 2014). A different levels of cheddar cheese were substituted by acid casein hydrolysate as shown in Table 2.

Table 2. Formulation of the processed cheese preparation with different acid casein hydrolysate levels (calculated as g/kg).

	Ingredients							
treatments	Cheddar cheese (gm)	Emulsifying salt (gm)	Skim milk powder (gm)	Butter oil (gm)	Potassium sorbate (gm)	Casein hydrolysate (ml)	Water (ml)	Total (gm)
Control	416.66	24.99	41.66	96.66	0.8	-------	419.7	1000,47
T_1	374.99	24.99	41.66	110.42	0.8	81.499	365.98	1000
T_2	333.33	24.99	41.66	124.99	0.8	162.17	312.17	1000
T_3	291.66	24.99	41.66	139.58	0.8	238.83	261.16	999.68
T_4	249.99	24.99	41.66	154.16	0.8	323.33	204.49	999.42
T_5	208.33	24.99	41.66	168.83	0.8	405.83	149.16	999.6

T_1= treatment has 10% casein hydrolysate, T_2= treatment has 20% casein hydrolysate, T_3= treatment has 30% casein hydrolysate, T_4= treatment has 40% casein hydrolysate, T_5= treatment has 50% casein hydrolysate.

A pre-blend of each of the six-process cheese spread formulations was prepared by mixing all the ingredients including natural cheese, other ingredients such as anhydrous milkfat, skim milk powder, emulsifying salt and water in a single screw cutting blade patch cooker at mixing speeds ranging from 70 to 300 rpm. The cooker started at 70 rpm for 20 min at room temperature to achieve a homogeneous paste. Thereafter, the temperature of the pre-blend was increased to 60°C and 2 N NaOH was added to adjust the final pH product approximately to 5.8 pH unit with constant stirring (70 rpm) for 4 minutes at the same temperature then, the temperature was raised to 85°C in 10 min and held for an additional 5 min. During heating and holding period of the process cheese manufacturing, the stirring speed was constant at 300 rpm. After cooking process, the cooked products were poured hot into 70 g, food commercial grade, plastic containers with a capacity of 80 g, which were lidded, cooled, and within 15 min placed in storage at 7°C. All the cooked process chesses were stored at 7°C until further analysis was completed. A 1.5 kg batch of each cheese sample was manufactured.

2-4- Casein hydrolysate preparation

Preliminary experiments were done to evaluate the sensory properties of bovine acid casein hydrolysate with trypsin and pepsin enzymes under suitable conditions at different times (0 to 1h). In this study, the produced hydrolysate with pepsin was prepared according to the method as described by Irshad *et al.*, (2015).

2-5- Determination of degree of hydrolysis (DH)

The degrees of hydrolysis of acid casein hydrolysates were measured according to Nielsen *et al.*, (2001).

2-6- Soluble Nitrogen at pH 4.60

Soluble nitrogen in the process cheese samples was determined according to the Association of Official Analytical Chemists (AOAC, 2012).

2-7- DPPH radical scavenging method

DPPH radical scavenging method for water soluble extract. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was performed by the method of Bhandari et al. (2010) to examine antioxidant activity of water-soluble peptides. All DPPH tests were carried out in triplicate.

2-8- Free amino acids analysis by CD-ninhydrin method

A sample of water-soluble extract at pH 4.6 was filtered through filter paper Whatman No 1 ant then through 0.45 µm, Multigrade, Glassfiber. The method of Folkertsma and Fox,

(1992) was followed. The optical densities of samples were measured at 507nm. The increases in optical densities were attributed to the increase in free amino acids.

2-9- Sensory evaluation:

The sensory properties of control and the experimental cheese were evaluated, by 10 trained panelists of staff members of Dairy department, Faculty of Agriculture, Minia University. according the scheme as described by Giri *et al.*, (2017).

2-10 -Statistical analyses

Statistical analysis of the data obtained was performed using statistical analyses One Way ANOVA with GraphPad Prism 5.0 (GraphPad Software, Inc).

RESULTS AND DISCUSSION

Chemical composition

The obtained results for the chemical composition of the process cheese after manufacture are presented in (Table 3) In general, the moisture, total solids, total proteins, fat and ash contents did not present significant differences ($p > 0.05$) between control and treated samples. Moisture contents ranged in fresh cheese from 55.38 in control to 56.68 in treated samples (40% casein hydrolysate addition) as shown in Table 3. These results are in agreement with *Code of Federal Regulations (2013),* which provides moisture content between 44% and 60% for process cheese spreads. The moisture contents are similar to that reported by,*Toro, et al (2016) and El-Garhi et al (2018)* for processed cheese spreads with values of 58.2%, 57.3 – 59.4 and 54.79% respectively. *Cunha and Viotto (2010) and El-Assar et al (2019)* reported that 62.77- 63.49% and 59,78 – 66.24% moisture in process cheese spread. This difference is correlated to the initial primary materials used in the manufacturing of the process cheese. Also, the moisture content of all treatments showed no significant changes during the storage period. The general trends of these results are in agreement with those reported by Mansour et al. (2011) and El-Garhi *et al.*, (2018).The protein content of control cheese was 13.49%, similar with that obtained result by Toro,et al.,(2016),El-Garhi et al.,(2018) . Dimitreli and Thomareis, (2008), prepared process cheese by mixing Gouda cheese with water, butter, and powdered skim milk and obtained a range of 12% to 15% for protein. However, the pH 4.6 soluble nitrogen (g/100 g total N) contents presented significant differences ($p > 0.05$) between control and treated samples as well as within the five treatment groups (the p-value is < 0.05). The control cheese had the lowest SN/TN values than all treated samples. For control sample it was 18.87 (represents 0.40% N). This result is lower than reported by Saad et al (2015) who found that 0.779% soluble N at pH 4.6. This difference may be attributed to the age and type of natural cheese used. In the present study young cheddar cheese was used (15 days at 25° C).

Fox et al (2017) stated that

the young Cheddar cheese (i.e., 15–35 days) has a pH 4.6-soluble N level of <12 % total N. However, the SN/TN for other treatments was significantly higher than the control cheese. The SN/TN increased gradually with increasing addition of casein hydrolysate. The highest value of SN/ TN was recorded by the treatment of 50% (26.95%, 0.49% N). During storage, the soluble nitrogen content significantly increased ($p > 0.05$) in all samples (control and treated samples) and proportionally with the extending the storage period. The increase was more obvious in control processed cheese where, the SN/ TN increased from 18.87% at zero time to 52.98% after 60 days storage. This trend is in accordance with that obtained by Saad et al (2015) who found that the soluble nitrogen increased during storage of process cheese for 90 days from 0.779 to 1.137%. These could be attributed to the residual activity of heat resistant proteinases present in the natural cheese used. Addition of emulsifier salts during processing increases the solubility of caseinate which becomes more susceptible to enzyme hydrolysis. El Dakhakhny and Dabour, (2016) claimed that the soluble nitrogen content in process cheese increased with increasing the pyrophosphate ratio in the emulsifying mixture. Additionally, the conversion of para-casein to a sodium para-k caseinate results in partial hydration and this is paralleled by large increases in the levels of water-soluble N, and N that is non-sedimentable on ultracentrifugation of process cheese (Fox et al 2004). The level of intact protein, as measured by the level of nitrogen insoluble at pH 4.6 (casein isoelectric pH), decreases concomitantly with paracasein hydrolysis.

The fat content was 25% for control and treated samples, accounting 56% fat in dry matter (FDM) (Table 3). No significant differences ($p > 0.05$) were observed between control and treated samples. According to Code of Federal Regulations CFR and FDA, (2006); Codex Alimentarius Commission; FAO/WHO, (2008) the minimum content of fat must be greater than or at least equal to 20% and moisture content lies between 44-60% in the final of process cheese spreads. FDM in the present study fulfills this requirement. These values are in accordance with that reported by Cunha and Viotto. However, El-Garhi *et al.,* (2018) who prepared process cheese spread from ultrafiltered milk retentates with 25% fat and 56% FDM. However, the fat content in the present study was higher than that stated by Sádlíková *et al.,* (2010)*;* Verma *et al.,* (2013) they showed that 21-22% and 19.8% fat respectively. The low-fat contents in their studies may be attributed to the higher levels of moisture which represent 58% and 57.51% respectively.

Table 3. Chemical composition of the process cheese fortified with different levels of casein hydrolysate during storage at 7±1°C for 60 days.

storage	Treatments	% T. S	%Moisture	%T. N	% S. N	SN/TN	%T. P	%Fat	FDM
FRESH	control	44.63	55.38	2.11	0.40±0.0005	18.87	13.49	25	56.02
	T_1	44.67	55.34	2.15	0.45±0.0005[a]	21.03	13.70	25	55.98
	T_2	44.50	55.51	2.14	0.46±0.0005[a]	21.59	13.65	25	56.19
	T_3	43.76	56.25	2.12	0.48±0.0005[a]	22.76	13.54	25	57.14
	T_4	43.33	56.68	2.18	0.48±0.0005[a]	22.62	13.92	25	57.70
	T_5	43.92	56.09	2.14	0.49±0.0005[a]	26.95	13.67	25	56.93
20days	control	44.47	55.54	2.12	0.52±0.0005	26.02	13.51	25	56.22
	T_1	44.55	55.45	2.15	0.55±0.0005[a]	27.36	13.71	25	56.12
	T_2	45.31	54.70	2.14	0.57±0.0005[a]	26.47	13.67	25	55.18
	T_3	44.90	55.10	2.15	0.59±0.0005[a]	23.92	13.73	25	55.68
	T_4	44.24	55.77	2.11	0.63±0.0005[a]	29.86	13.46	25	56.52
	T_5	44.06	55.95	2.12	0.76±0.0005[a]	35.68	13.52	25	56.75
40 days	control	44.23	55.77	2.12	0.65±0.0008	34.63	13.54	25	56.97
	T_1	42.65	57.35	2.15	0.68±0.0006[a]	30.28	13.72	25	58.62
	T_2	43.95	56.05	2.18	0.70±0.0006[a]	31.28	13.92	25	56.89
	T_3	43.97	56.04	2.15	0.74±0.0005[a]	32.66	13.74	25	57.77
	T_4	43.39	56.61	2.14	1.01±0.0005[a]	47.19	13.63	25	57.61
	T_5	43.62	56.38	2.12	1.03±0.0005[a]	48.49	13.54	25	57.32
60 days	control	44.95	55.05	2.13	0.93±0.0006	46.90	13.57	25	56.51
	T_1	44.74	55.27	2.14	0.96±0.0006[a]	45.67	13.64	25	56.33
	T_2	45.02	54.99	2.14	0.98±0.0006[a]	44.65	13.65	25	55.54
	T_3	45.05	54.95	2.14	1.00±0.0006[a]	43.67	13.65	25	56.16
	T_4	44.65	55.35	2.15	1.13±0.0005[a]	52.74	13.72	25	56.22
	T_5	43.02	56.98	2.18	1.16±0.0005[a]	52.98	13.91	25	58.58

T.S is total solids; T N is total Nitrogen; T P is total proteins; S N is 4.6 pH soluble Nitrogen; FDM is fat in dry matter; T_1= treatment has 10% casein hydrolysate, T_2= treatment has 20% casein hydrolysate, T_3= treatment has 30% casein hydrolysate, T_4= treatment has 40% casein hydrolysate, T_5= treatment has 50% casein hydrolysate, a is significant difference with control.

DPPH

The radical scavenging activity of process cheese with addition of different levels of acid casein hydrolysate (0 – 50%) is shown in (Table 4). The results showed that a steady increase in radical scavenging activity of process cheese with the addition levels. A significant difference ($P < 0.05$) recorded between radical scavenging activities among all the experimented samples. Increasing the concentration of casein hydrolysate resulted in increased DPPH radical-scavenging activity. In fresh cheese, the DPPH radical-scavenging activities of the cheeses fortified with 0, 10, 20, 30, 40 and 50% casein hydrolysate were 16.59, 17.35, 17.81, 18.72, 19.48 and 22.98, respectively. These results were in consistent with those reported by Irshad *et al.*, (2015) they determined the DPPH radical-scavenging activities of the casein hydrolysate by trypsin alone and with a combination of trypsin and pepsin at different time intervals. Their results showed that the DPPH radical-scavenging activities increased in case of casein hydrolysate by pepsin from 1.2 % to 13.1% at 0.5 and 4 h hydrolysis, respectively.

However, in the case of bovine casein hydrolysate by pepsin the radical scavenging activity showed slightly decrease as compared to individual tryptic hydrolysis. They attributed the radical scavenging activity of pepsin-hydrolysate casein to the formation of N-terminal of aromatic amino acids like Phe, Trp and Tyr and formation of small bioactive peptides below 1 kDa (hexapeptide Leu-Pro-His-Ser-Gly-Tyr with a molecular weight of 672 Da) which exhibited the highest antioxidative activity. Similar findings were observed by Liu et al (2009) who found that the radical scavenging activity of porcine plasma protein hydrolysates increased with the increase in degree of hydrolysis and the highest antioxidant activity has been showed after 5 h hydrolysis.

Moreover, Díaz and Decker, (2004) attributed the antioxidant activity of bovine casein hydrolysate to the formation of Caseinophosphopeptides (CPP) which able to bind prooxidant metals such as iron. They further indicated that the thiobarbetoric acid reactive substances (TBARS) formation was inhibited 75, 39, and 17% by 0.5% enriched CPP, casein hydrolysates, and low molecular weight casein hydrolysates, respectively, after 4 days of storage. Additionally, Irshad *et al.*, (2015) suggested that pepsin has specificity towards N-terminal of aromatic acids such as Ph, Trp and Tyr and the phenyl groups of newly released peptides aromatic amino

acids (hydrophobic amino acids) whereas trypsin has specificity towards basic amino acids like arginine and lysine (hydrophilic amino acids). Both types of amino acids have the affinity to scavenge free radicals. The antioxidant capacity of cheddar cheese increased significantly with the progress of ripening time, the maximum of antioxidant capacity was reached after 3 months and 1 – 4 months for winter and summer cheese respectively and then decreased but, this decrease was not statistically significant (Lee *et al.,* (2015) and Revilla *et al.,* (2016).

Martín-del-Campo *et al.,* (2019) stated that 26% DPPH-scavenging activity of hydrolysed whey concentrate by trypsin at 52 °C and a pH of 8.2. During storage at refrigerated temperature (7°C ± 1°C) for 60 days, the radical scavenging activity of process cheese significantly increased ($P < 0.05$). Results in Table 4 showed that the radical scavenging activity increased from 16.59, 17.35, 17.81, 18.72, 19.48 and 22.98 in fresh to 55.926, 55.926, 58.585, 64.44, 68.889 and 71.667% after 60-day storage for control and treated samples with 10, 20, 30, 40 and 50% casein hydrolysate respectively. This may be attributed to the increase of protein hydrolysis during storage where, the pH-soluble nitrogen significantly increased during storage (Table 3). Revilla *et al.,* (2016) Öztürk and Akin, (2017) showed that the changes in the antioxidant activity of Tulum cheeses (soft white cheese) were related to the rate of formation of soluble peptides (proteolysis) during storage period.

Each value is the average of three analyses.

CD-ninhydrin

All amino acids in food protein (20 amino acids) react with ninhydrin giving purple color except amino acid proline gives yellow color and cysteine exhibits very low purple color. Therefore, determination the intensity of purple color can give the insight into the progress of proteolysis during storage, particularly the release of small peptides and amino acids (Radeljević *et al.,* 2013). Proteolytic activity (expressed as free amino groups) was estimated in process cheese while fresh and during storage for 60 days at 7°C ± 1°C. The results were represented as the increase in optical densities (O D) at 507 nm after reaction with Cd-ninhydrin (Folkertsma and Fox, 1992). The results in Fig (1) showed that the intensity formed color in fresh cheese was significantly ($P>0.05$) higher in cheeses manufactured with the addition of casein hydrolysate in comparison with control samples. Moreover, the optical densities significantly increased ($P>0.05$) with the increase of casein hydrolysate level. The O.D values were 0.251, 0.467, 0.533, 0.598, 0.693 and 0.723 for control, 10, 20, 30, 40, and 50% casein hydrolysate addition. This is not surprising because the casein hydrolysate contains high percentage of small peptides and free amino acids (Table 3). During storage at refrigerated temperature (7°C ± 1°C) for 60 days, the optical densities of process cheese samples significantly increased ($P < 0.05$).

Table 3. The radical scavenging activity of process cheese manufactured with different levels of acid casein hydrolysate during storage for 60 days.

Storage	**Control**	**Casein hydrolysate (%)**				
		T1	T2	T3	T4	T5
Fresh	16.59±0.005	17.35±0.005[a]	17.81±0.006[a]	18.72±0.005[a]	19.48±0.005[a]	22.98±0.005[a]
20 days	24.93±0.006	28.12±0.006[a]	30.43±0.006[a]	31.52±0.005[a]	33.33±0.005[a]	46.81±0.005[a]
40 days	27.03±0.006	34.42±0.005[a]	38.33±0.005[a]	48.48±0.006[a]	51.88±0.005[a]	55.94±0.005[a]
60 days	55.93±0.006	55.93±0.006[a]	58.59±0.005[a]	64.44±0.005[a]	68.89±0.006[a]	71.67±0.006[a]

T_1= treatment has 10% casein hydrolysate, T_2= treatment has 20% casein hydrolysate, T_3= treatment has 30% casein hydrolysate, T_4= treatment has 40% casein hydrolysate, T_5= treatment has 50% casein hydrolysate, a is significant difference with control.

Results in Fig. 1 showed that the optical densities increased from 0.251, 0.467, 0.533, 0.598, 0.693 and 0.723 in fresh cheese to 0.976, 0.986, 1.023, 1.137, 1.164 and 1.203 after 60 day storage for control and treated samples with 10, 20, 30, 40 and 50% casein hydrolysate respectively. This may be attributed to the increase of protein hydrolysis during storage where, the pH-soluble nitrogen significantly increased during storage (Table 3). Similar observation was reported by Radeljević *et al.,* (2013) who stated that the free amino acids of Krk cheese (hard Croatian cheese) significantly increased during storage period (0 -120 days). They attributed the increase in free amino acids to the increase of nitrogen -containing substances, which include free amino acids, mostly occur by the action of indigenous heat resistant proteolytic enzymes (i. e plasmin), starter culture enzymes and non-starter culture enzymes. In relation to the optical determined on the 60th day of storage of process cheese, the highest increase is visible in the first 20 days of storage. Thus, the concentration up to day 20 increased from 0.251, 0.467, 0.533, 0.598, 0.693 and 0.723 fresh control cheese, 10, 20, 30, 40 and 50% casein hydrolysate to 0.818, 0.914, 0.923, 0.935, 0.939 and 0.947, respectively. This may be due to the intensive activity of residual enzymes during the first 20 days of storage which is expressed as the increase in the pH-4.6 soluble nitrogen (Table 3). Hayaloglu *et al.,* (2005) found that the concentrations of total free amino acids in cheeses, as measured by the Cd-ninhydrin method, increased gradually until 60 day, and then sharply at 90 day of storage. Also, they found correlation between the increase in phosphotungstic acid-soluble nitrogen (PTA-SN) and water-soluble nitrogen from one side and concentration of total free amino acids from another side. These findings agree with those reported by Tokusoglu and Swanson, (2014) who reported that the concentration of free amino acid in water soluble fraction extracted from cheese slurry made from raw milk was higher than those from pasteurized or pulsed-electric field milk.

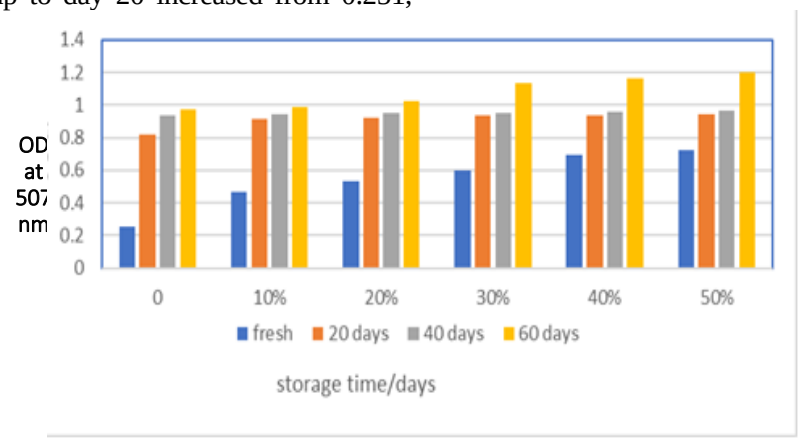

Fig. 1. Average concentration of total free amino acids expressed as optical density at 507nm of process

Sensory Evaluation

The mean sensory score of ten judges for three replicates of the process cheeses shown in (Figs 2-8). The level of casein hydrolysate addition significantly influenced (P <0.05) all the evaluated parameters. Treatment 10% casein hydrolysate with mean score of 6.63, 6.63, 6.38 and 6.33 for appearance, aroma, taste and total score, respectively was significantly greater than all other samples. Also, sample treated with 20% recorded (6.5, 6.38, 5.88 and 6.25) were not significantly different from those of control (6.5, 6.25, 5.75 and 6.14) for the same parameters.

However, cheese made with 40 and 50% casein hydrolysate received significantly (P < 0.05) lower scores than the other cheeses with respect to all measured parameters. In general, no significant differences (P <0.05) were observed between control and treated cheese with 10, 20 and 30% casein hydrolysate for total scores therefor, these samples can be grouped together. During storage periods there were slight changes in all parameters until 60 days storage. Treatments with 10, 20 and 30% casein hydrolysate addition possessed quality more or less similar to control samples until 60 days storage. Samples treated with 40 and 50% casein hydrolysate addition received lower scores and unacceptable products. After 60 days storage at refrigerate temperature, treatment with 50% casein hydrolysate showed water phase separation as shown in Fig. 9.

Conclusion

The effect of addition acid casein hydrolysate on the physicochemical properties, sensory characteristics, and antioxidant activities of process cheese spread cheese was investigated. It can be concluded that the total free amino groups content increased with increasing concentration of acid casein hydrolysate. The radical scavenging activities (antioxidant activities) of process cheese significantly increased with addition of different levels of acid casein hydrolysate. Furthermore, the cheese fortified with 10, 20 and 30% were valuable in view of sensory and antioxidant effects. Therefore, these results showed that supplementation with acid casein hydrolysate has good potential for use in process cheese for enhancing antioxidant effects.

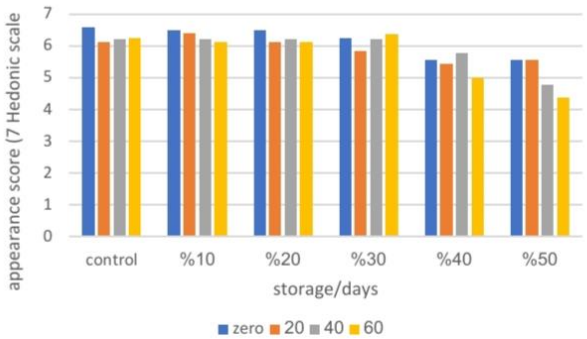

Fig 2 . Appearance scores (7 Hedonic Scale)

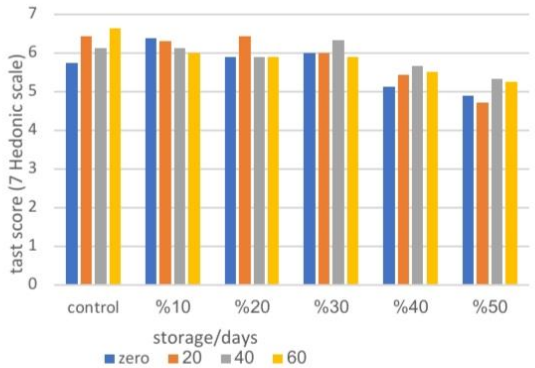

Fig 4. Taste scores (7 Hedonic Scale)

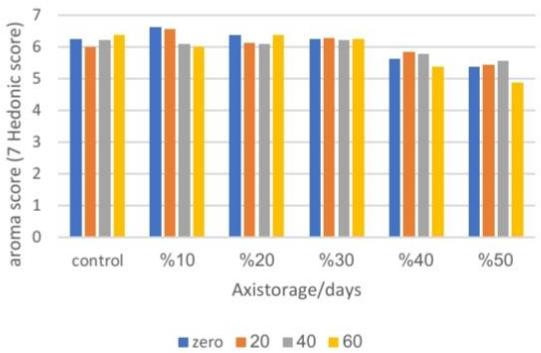

Fig 3. Aroma scores (7 Hedonic Scale)

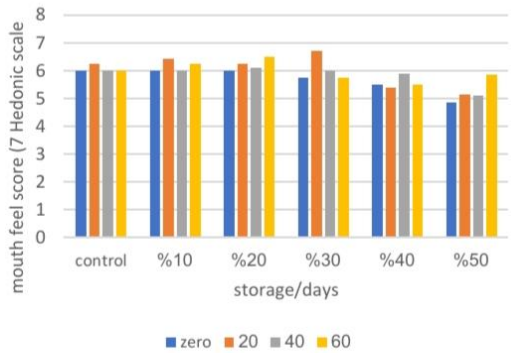

Fig 5 . Mouth feel scores (7 Hedonic Scale)

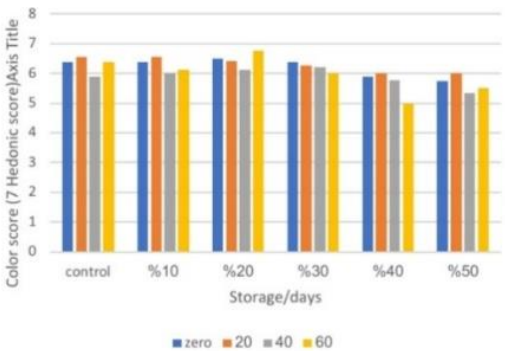

Fig 6 Color scores (7 Hedonic Scale)

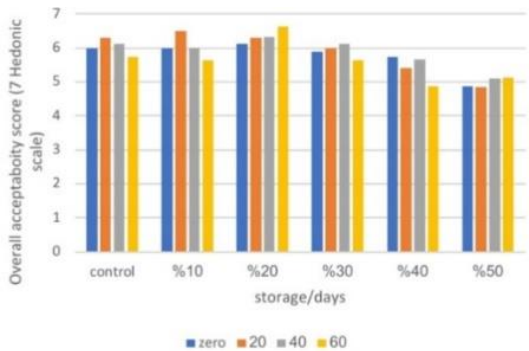

Fig 7 Overall acceptability scores (7 Hedonic Scale)

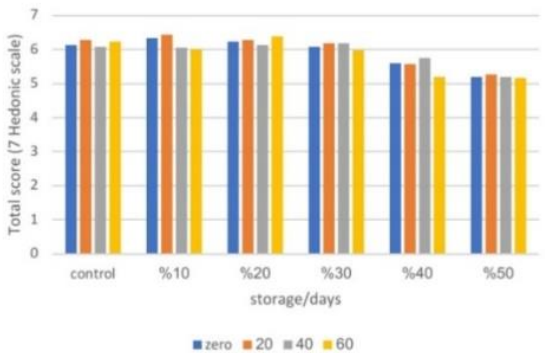

Fig 8 Total scores (7 Hedonic Scale)

Fig. 9. shows water phase separation after 60 days storage for 50% treatment.

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صفات مضادات الأكسدة للجبن المطبوخ القابل للفرد المدعم بالكازين الحمضى المتحلل

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أجريت هذه الدراسة بهدف انتاج جبن مطبوخ قابل للفرد ذات محتوي مرتفع من مضادات الاكسدة و البيبتيدات و الاحماض الامينية الحرة ذات خواص حسية عالية الجودة. تم تحضير خلطات الجبن المطبوخ القابل للفرد المصنع من الجبن الشيدر(تخزين 15 يومًا) و إضافة الكازين الحمضي المتحلل باستخدام البيسين بمستويات مختلفة (0 ، 10 ، 20 ، 30 ، 40 و 50٪). تم تقدير التركيب الكيميائي ونشاط مضادات الأكسدة والمجموعات الأمينية الحرة والخصائص الحسية للمنتج.

أظهرت النتائج زيادة نشاط مضادات الأكسدة للجبن الناتج مع زيادة تركيز متحلل الكازين من 55,93 الى 55,93 و 58,59 و 64,44 و 68,89 و 71,67 بعد التخزين لمدة 60 يوم . أيضًا تم تقدير مجموعات الأحماض الأمينية الحرة في الجبن المطبوخ عقب التصنيع وأثناء التخزين لمدة 60 يومًا على 7 درجة مئوي (± 1 درجة مئوية)، على طول موجى 507 نانوميتر وتم تمثيل النتائج على أنها زيادة في الكثافة الضوئية . زادت هذه القيم من 0.976الى 0.986 و 1.023 و 1.137 و 1.164 و 1.203 بعد تخزين لمدة 60 يومًا بنفس الترتيب. علاوة على ذلك، خضع منتج الجبن للتقييم الحسي من حيث المظهر والرائحة والطعم و واللون والقبول العام. واظهرت النتائج انه لا يوجد فروق ما بين العينة الكونترول والعينات المعاملة بـ 10 و 20 و 30 ٪ من الكازين المتحلل و لا توجد فروق ذات دلالة إحصائية. وان الجبن المصنوع بإضافة 40 و 50٪ من الكازين المتحلل لهما صفات غير مقبولة ولوحظ انفصال للماء بعد 60 يومًا من التخزين في العينة المعاملة بإضافة 50٪ كازين متحلل.

لذلك توصى الدراسة بأن أفضل النسب المضافة من الكازين المتحلل هي لإنتاج جبن مطبوخ قابل للفرد عالي الجودة هي 10، 20، 30 %