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DERIVED PEPTIDES FROM MILK PERMEATE AS A HEALTH INGREDIENTS: PROPERTIES AND APPLICABLE ASPECTS

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ABSTRACT

The herein study aimed to isolate natural antioxidant biologically active peptides from permeate and tested their positive effect against oxidative rancidity in edible oils in comparison with synthetic antioxidant namely butylated hydroxylanisol(BHA) .The purification of the reconstituted lyophilized permeate from minerals was performed by loading on sephadex G.10 column and isolation of peptides was carried out using electrophoresis method.The obtained results revealed that permeate fractioned on 12.5% acrylamide gel into two effective peptides of about 2000and 6600 Daltons for B1and B2 respectively. Variation in m.w between the obtained two peptides may be due to the variation of amino acids concentration namely aspartic, therionine, arginine, valine, methionine, phenylalanine, histidine, tryptophane, lysine which they are composed. The total antioxidant activity (TAA) of 100 µg peptide of both B1 and B2 is equivalent to 9.46 and 7.94 µg ascorbic acid, respectively. Stability results of obtained peptides showed that frying time had no effect on both peptides. Data showed that the role of derived peptides in reducing the oxidative rancidity of corn oil and butter oil in comparison with BHA and control .Incorporation of B1and B2 with sun flower oil reduced the effect of polymers content being 25% and 19%, respectively, after 6 days .Rancimat test reveled that both peptides are effective on the stability of oils similar to that obtained by BHA. The herein study proved that the isolated peptides are effective against rancidity in oils, further are recommended to utilize it in nutraceuticals and foods.

Keywords: Permeate, Isolated peptides, antioxidant activity, heat stability, lipid oxidation, polymer, rancimate.

INTRODUCTION

Permeate is the major by-product obtained from ultrfiltration technique used in cheese manufacture.. Permeate contains about 5.8-6.0 % total milk solids. Permeate is a source of high quality soluble proteins, lactose, vitamins and minerals that are important to the human health. Permeate resembles a problem for environmental pollution. However, because of its high organic content its disposal can pose environmental problems. Milk permeate showed higher BOD value (about 60,000 mg/l) and, therefore, they cannot be drained without a treatment. Thus milk permeate could be used for the manufacture of some food products such as ice-creams, cooked products, bakery blends, fermented beverages, or utilized in the production of useful products such as lactic acid, polyhydroxy alkanoate, gluconic acid, citric acid …etc. (Abd El-Khair, 2009). Various compounds namely, antioxidant (natural and synthetic) added to certain foods to retard

outoxidation . It is necessary to utilize antioxidants to lipid before use in food production (Farag, *et al* ., 2003)

In recent years, functional foods have broken their way into the food industry due to the huge awareness of consumers referring to the relationship that exists between diet and health. Among the functional ingredients, defined as those components which, incorporated into food, exhibited specific biological activities which go beyond a many nutritional role, one of those was refered to their diversity and multifunctionality, being the bioactive peptides. These peptides are inactive fragments within the precursor protein, but which, following their release by means of in vivo and/or in vitro hydrolysis processes exert different physiological functions in the body. Since their discovery in 1979, peptides derived from food proteins with different biological activities: antimicrobial, antihypertensive, immunomodulating, antithrombotic, opioid, antioxidant, etc. have been described (Rival, *et al*., 2001; Suetsuna, *et al*., 2000 and Hernandez-Ledesma *et al*., 2005). These peptides have a potential use in foods and/or pharmaceuticals

Recent studies have shown that antioxidative peptides can be released from caseins by degradative enzyme, even so by digestive,or by fermenting lactic acid bacteria strains (Korhonen and Pihlanto Leppala ,2006).

The production of the antioxidative bioactive peptides from milk permeate would make it possible to find new uses for this by product beyond its conventional nutritional value, including nutraceutical products. Refering to its effects for healthy development and, high-quality foods, contributing to make the best use of milk by products which have more highly valued.

Therefore, in this study we used buffaloes milk permeate as a cheese industry waste to isolate antioxidative biologically active peptides and tested their activities under oxidation system in comparison with those of synthetic antioxidant Butylated hydroxyanisol (BHA). In addition, the purified peptides effects against oxidation under some technological parameters were determined.

MATERIALS AND METHODS

Fresh buffalo's milk permeate was obtained from Animal Production Research Institute Pilot unit, Agric Res .Cent. , Dokki, Giza,Egypt.

Ammonium persulphate was obtained from Lambmerk (India), Brilliant blue R was obtained from Brix Wath Northants U.K., Bromophenol blue was obtaind from Fischer chemical , Fischer Scientific company , Germany.Glycine obtained from El- Nasr Pharmaceutical, Glycerol obtained from El-Nasr pharma, Egypt. N, N-Methylene bisacrylamide GR obtained from LOBA chemie (India). ,N.N.N.N tetrmethylenodiamine(Temed) obtained from medex : medical exportco.LTD (USA) ,Mercaptoethanol from MP Biomedicals,LLC Germany, , Sodium Lauryl Sulphat was obtained from RFCL Limited (New Delhi – India), Tris (hydroxy methyl amino methane) was obtaind from BDH Laboratory,England, low range protein ladder (1.7-40 KDa) thermo Scientific, USA. Butylated hydroxyanisol (BHA) was obtained from Ranbaxy Fine Chemical Limited (New Delhi India), Sephadex G-10 obtained

from Pharmacia Sweden. Polysorbatee 20 obtaind from medical export company, UK.

Permeate was lyophilized by lyophilizer apparatus (Model Snijders Scientific b.v.Holland Model: LYSFM. The lyophilized permeate was reconstituted with deionized water in ratio of 1:2 w/v. Chemical analysis of milk permeates: Lactose, Total nitrogen , Ash and moisture were detrmined according to AOAC (2007). Amino acids in derived peptides were determined according to James, et al., (1988). Rancimat method was carried out according to Mendez,*et al*,. (1996).Acceleration test was carried out according to Sherwin and Jaocs.(1968) Peroxide value was determined according to AOAC (2007). Thiobarbituric acid (TBA) was determined according to Pearson (1976). Polymer content was determined according to Pei-Fen and Nawar (1986). Antioxidant activity was determined according to Nogala,*et al*,.(2005) The role of peptides in the stability of sun flower oil upon frying treatment was determined according to Francisco and Zamora (2006).

For the Purification of peptides, 5 ml of permeate solution was eluted on Sephadex G-10 column (lenth 52 cm , diameter 2 cm) to separate the lactose and minerals using phosphate buffer pH 6.8 and the peptide fraction were collected and the nitrogen was determined in fractions according to Lottspeich (1998). The solution was separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method according to (Lammli, 1970).

The sample were boiled at 95^{o}C for 5 min after mixing with 5X sample buffer at ratio of 1:1 (V:V). The resolving and staking gel were prepared according to Lammli, (1970). 20 ul of sample were loaded separately into slots, vertical gel electrophoresis (V15-17, Biometra, Germany), which used first at 80 Volt until the bromophenol blue dye reached the resolving gel and continued at 150 Volt for 5 hr. The peptide bands were observed after staining the gel with Commassie Brilliant blue (R-250) about 12 hr and incubated in destaining buffer at RT until the background was colorless then photographed.

Peptide isolation:

Isolation of peptide was carried out by using a scalpel, the portion of gel containing the band was cut out without staining using the marker of peptides. Divided it into pieces of about 2mm square, and the pieces were collected in Eppendorf tubes. Another portion of gel ,about the same size as the first ,from a region contained no peptides (blank) was cut out and divided up as described above, and treat it simultaneously with the sample containing the peptide. The resulting bands (2cm length) were soaked in phosphate buffer pH (6.8) for 12 h using receiver tubes. The gel was removed and the peptide solution was loaded on fraction collector apparatus (Bio. Rad –model 2110) for desalting the sample in about 100 test tube using Sephadex G 10.

For studying the effect of frying time on the stability of isolated peptide incorporated with sunflower oil, the method of Francisco and Zamora (2006) was used as follows: Sunflower oil incorporated with B1 and B2 in the presence of emulsifing agent (Tween 20), used 1 ml peptide solution (equivalent to 100 ug)/100g of oil for frying at interval times (0, 5, 15 min)

during frying, oils were taken for extraction of peptides using acetone, all samples taken were applied for isolation peptides by electrophoresis.

RESULTS AND DISCUSSION

Lyophilized buffaloes milk permeate contained 85% lactose, 3% soluble nitrogen, 7.5% ash and 4.5% moisture. The peptides present in delactosed permeate were fractionated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) after desalting and delactosed using Sephadex G-10 upon different concentrations of acrylamide being (7.5, 10, 12.5 and 15%). The permeate in 12.5% acrylamide was subsequently fractionated into three peptides. Two peptides were a mixture of (B2) had a molecular weight of about 2000 Dalton and one peptide (B1) had a molecular weight of about 6600 Dalton were used according to their antioxidant activity in vitro experimental application (Fig. 1)

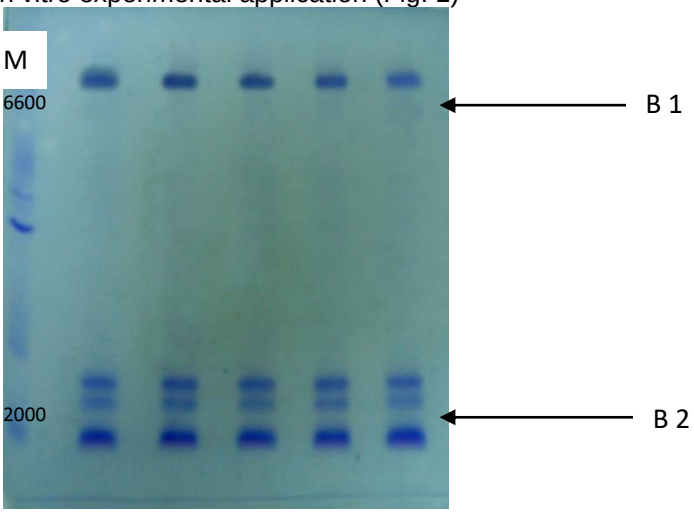

Fig. (1) : SDS-PAGE separation of peptides permeate.

Generally, the gel acrylamide ratio used has notable effect on separation of peptide bands of permeate. This finding might be due to the pore size of resolving gel selected based on the molecular weight of bands by altering the concentration of acrylamide in solution which are usually separated on resolving gels that contain 4-15% acrylamide. Acrylamide concentration of 15% is often used to separate peptides with molecular weights below 50,000 Daltons (Nielsen, 1998). The quality of peptides separation for the aforementioned two cases must be optimized in between the mixture of sample used and the concentration of gel in solutions (Warlker, 2002).

In respect to the physical and chemical properties of obtained derived peptides which were analyzed for the identification of their characters are presented in Table (1)

Table (1): Some characters of the isolated peptides from buffalo's milk permeate.

Characters	Specifications	
	Peptide (1)	**Peptide (2)**
Color	Colorless	Colorless
pH	7.2	7.3
Refractive index	1.5	1.5
Solubility in water	Soluble	Soluble
Solubility in hydrochloric acid buffer (pH 2)	Soluble	Soluble
Solubility in acid phthalate buffer (pH 4)	Soluble	Soluble
Solubility in phosphate buffer (pH 6,7)	Soluble	Soluble
Molecular weight	6600D	2000D
Specific gravity	1.05	1.04

These results revealed that insignificant variation in characters between the two peptides under study except in molecular weight which may be due to the variation of amino acids concentration in both peptides.

Amino acids composition of isolated peptides (B1 and B2) from milk permeate determined by HPLC – instrument are shown in Table (2).It could be stated that the presence of some of these amino acids has been considered to be essential in order for the antioxidant activity as exerted, and this activity has taken on major importance in the antioxidative properties of histidine containing peptides (Chen et al., 1998). It was clear that the quantitative order of the amino acid composition of the peptide B1 was Tryp> Val> Lys >Meth> His > Phen>Asp > Thr>Arg and the peptide B2 Meth> Tryp>His>Lys> Val>Asp>Phen> Ther. The antioxidative activity of histidine-containing peptides has been reported (Uchida and Kawakish, 1992), and their activity might be from hydrogen-donating ability, radical trapping ability or the metal ion-chelating ability of the imidazole group (Chan and Decker, 1994).. In addition, the structure or amino acid sequence of the peptide might play an important role in the antioxidative activity

Table (2): Amino acids (AA) analysis of isolated peptides from buffaloes milk permeate

Amino acid	**AA % from TN of B1**	**AA % from TN of B2**
Aspartic	(1.29)	(6.5)
Therionine	(0.01)	(0.07)
Arginine	(0.04)	(--)
Valine	(17.7)	(7.5)
Methionine	(16.0)	(31.06)
Phenylalanine	(8.5)	(5.38)
Histidine	(12.2)	(9.89)
Tryptophane	(23.9)	(25.4)
Lysine	(16.3)	(8.7)
TN: Total nitrogen		

Table (3) and Fig (2) show the obtained data concerning the activity of isolated peptides (1 ml) used in in concentration equivalents to 100 µg protein comparing with standard ascorbic acid.(Table 4 and Fig 2).

Table (3): Tptal antioxidant activity (TAA) of the isolated peptides from buffalos milk permeate

	Conc. (µglml)	antioxidant activity %
Ascorbic acid	**10**	**44.2**
B 1	**9.46**	**41.8**
B 2	**7.9.4**	**35.1**

Table (4) : Standard ascorbic acid

Ascorbic acid (µg/ml)	*Antioxidant activity %*
0	0
5	21.8
10	44.2
15	62.7
25	88.8
50	95.7
75	97.6
100	98.5

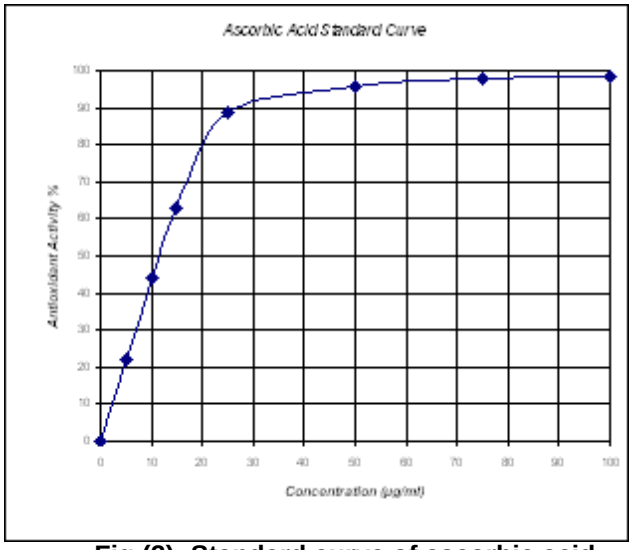

Fig.(2): Standard curve of ascorbic acid

The obtained data revealed that the total antioxidant activity (TAA) of 100 µg proteins of both peptides 1 and 2 is equivalent to 9.46 and 7.94 µg ascorbic acid respectively. The preceding data are in agreement with Hernandez- Ledesma, *et al*,. (2005) who found that whey hdrolysates could be suitable as a natural ingredients in enhancing antioxidant properties of functional foods and preventing oxidation reaction in food processing .also Lopez, *et al,*. (2007) found that casein hydrolysates may be a source of antimicrobial, antioxidant, by exhibition the oxygen radical absorbance capacity, seven times higher

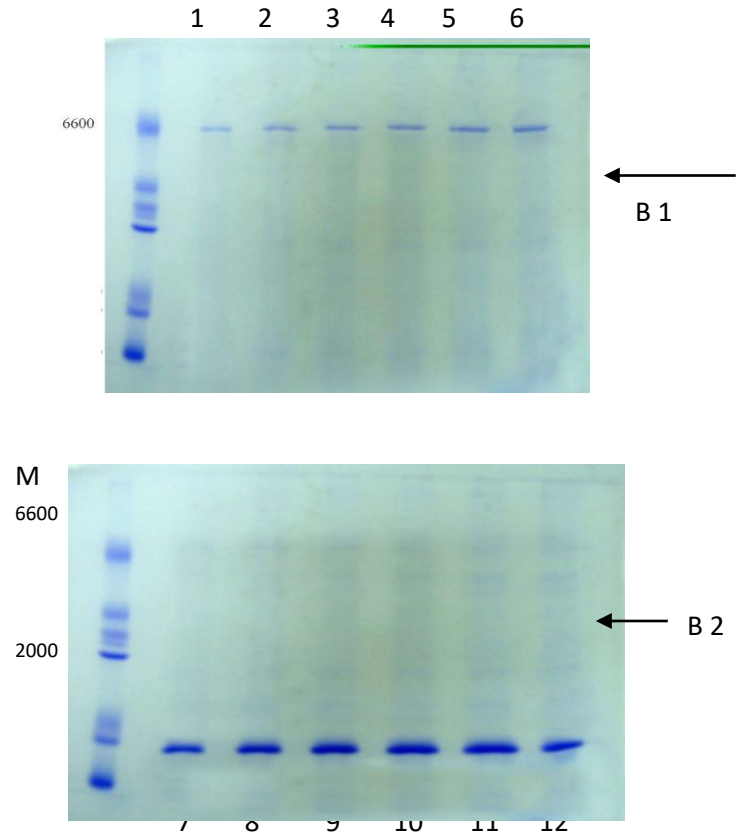

Fig. (3): Effect of frying time on the stability of isolated milk permeate peptide1 in sunflower oil. No:1,2,3 (shrimp at 0, 5 and15 min respectively).No:4,5,6 (potato fingers at 0,5 and 15 min respectively)

For detecting the heat stability, as deep fat frying normally carried out at high temperature (between 160ºC -180ºC) the presence of air, moisture, the food component affects the physical and chemical characters. Figs (3 and 4) show the effect of using isolated permeate peptides on their stability during frying shrimp and potato fingers at interval time (0, 5 and 15 minutes). It could be seen obviously from the obtained data that frying under the tested conditions had no effect on the stability of both peptides 1(6600d) and 2(2000d). The obtained data are in agreement with that obtained by Francisco and Zamora (2006) who clarified the role of peptides in the stability of edible oils.

As for rancidity reactions during acceleration test of corn oils incorporated with peptides under study, the peroxide values of an oil or fat has been carried out as a measurement of oxidative rancidity which are an intermediates in the auto-oxidation reaction.

As regards peroxide value which is a marker for the development of oxidation rancidity and realistic determination of stability in oils, acceleration method was used, to achieve this target. Fig 4 shows the data concerning the development of peroxide values at interval periods every three days by using 5 gm for each sample of corn oil up to 21 days at 65ºC during the course of treatment, upon BHA ,B1 and B2 manipulation for corn oil in comparison with control ones. The results showed that corn oil had a value of peroxide being 2.5 at 0 time determination and was found to be 22 and 10 after 15 and 21 days respectively. It is clear from this Table result that the increase was about 2.2 and 9.3 for BHA incorporated with corn oil at 0 times and after 21 days respectively corresponding to control. Similar effect was detected when corn oil was incorporated with B1 or B2 during the course of treatment, where the peroxide values were found to be 3 , 2.5 and 9.8 , 9.5 at 0 time and after 21 days for B1and B2 respectively.

It could be noticed that gradually increases into peroxide value up to 21 days for BHA, B1 and B2 respectively. Control data revealed that peroxide are formed during the acceleration test followed by decompose at the end of treatment (Sultana,etal 2007). Similar trend of results can be seen from Fig (6) concerning butter oil, under the same conditions of treatment where the peroxide values were determined by using 5 gm butter oil for each sample up to 36 days. Peroxide value of butter oil increased gradually up to 20 after 24 days for control , while incorporation BHA and derived peptides showed an increase gradually of peroxide value being 2.5,3,3.5 for initial time and 9,9.1 9.8 after 36 respectively in comparison with control one.

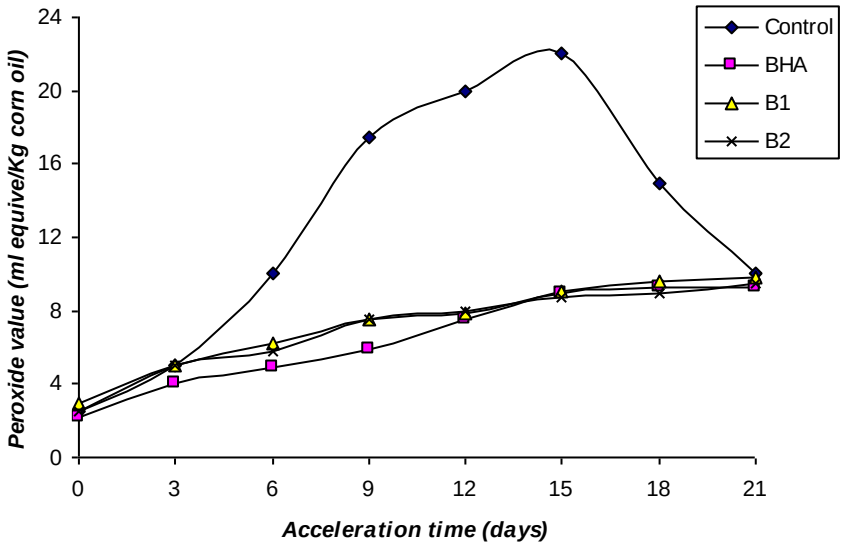

Fig 4: Peroxide values (ml equiv/Kg corn oil) upon addition of BHA and isolated peptides during acceleration test.

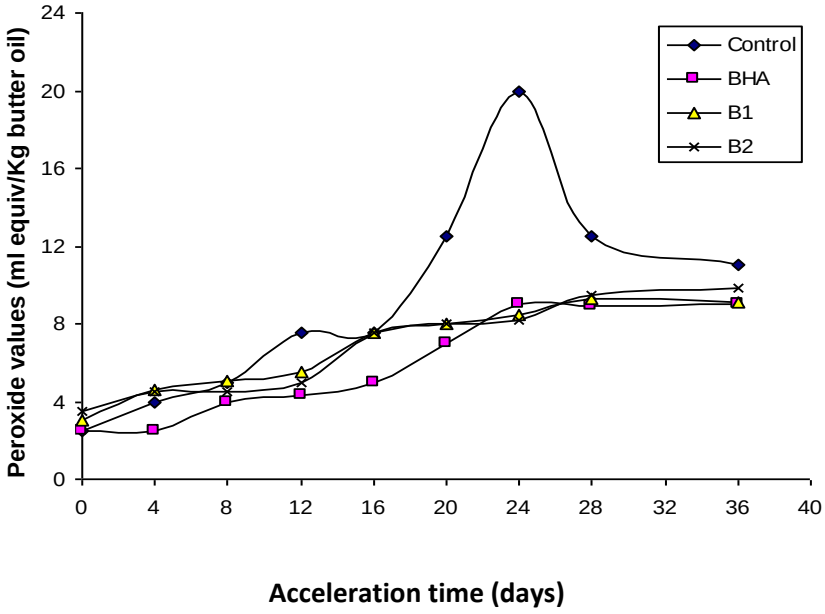

Fig 5: Peroxide values (ml equiv/Kg butter oil) upon addition of BHA and derived peptides during acceleration test.

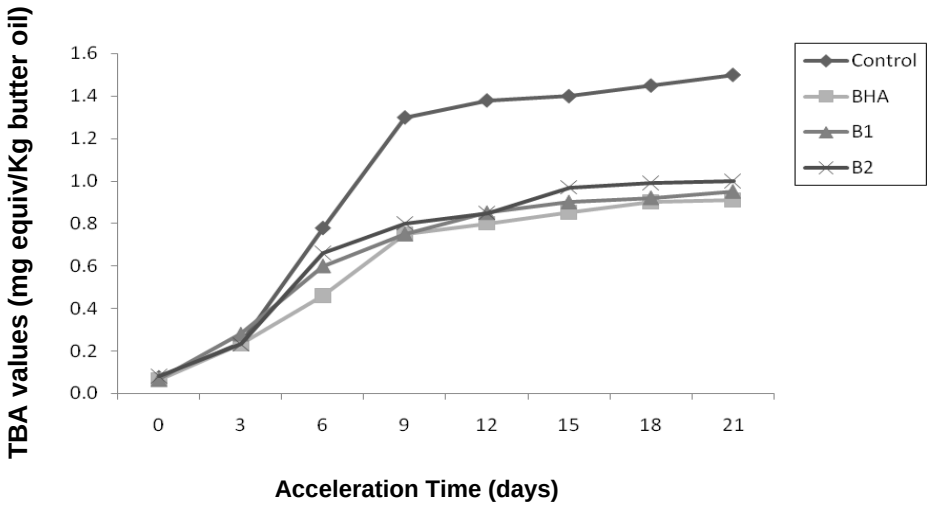

Fig 6: TBA values (mg equiv.kg corn oil) upon addition of BHA and derived peptides during acceleration test.

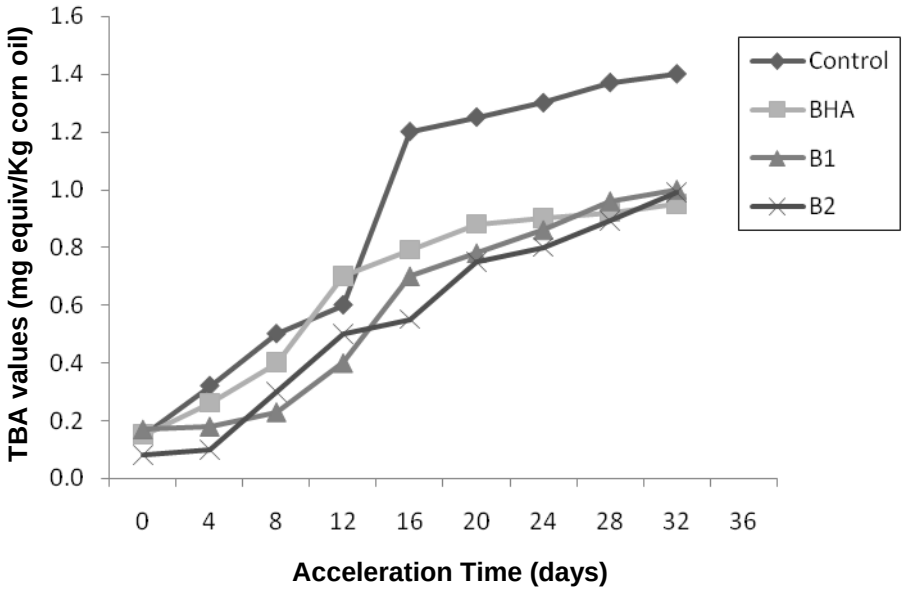

Fig. 7: TBA values (mg equiv/Kg butter oil) upon addition of BHA and derived peptides during acceleration test..

Fig (6): shows the antioxidant effect of milk permeate isolated peptides and synthetic BHA separately incorporated with corn oil. The obtained control data revealed that the TBA values increased gradually during the As shown from Fig 4 and 5 peroxides were increased during the acceleration time at 65ºC and after that reduced for control. Differences in obtained results can be drown regarding the variation in time needed to occur in between oil and butter oil may be due to the variation in fatty acid content of the two products.

TBA determination is a method for the analysis of certain carbonilic oxidation products in stored fats and oils which causing their rancidity.
acceleration test up to 0.078, 1.5 at (0 and after 21 days). BHA, B1 and B2 causes a decrease in TBA values being 0.91, 0.95, 1.0 respectively in comparison with control.

As shown in Fig (7): Similar tend with results concerning butter oil to that obtained with corn oil. (Park,*et al*,. 2011).It can be assumed from the preceding results that the antioxidant role of derived peptides namely B1and B2 in reducing the rancidity of butter oil in comparison with control.

Effect of isolated peptides from buffalo's milk permeate on polymer content in frying sunflower oil was examined. Fat changes during frying were attributed to the formation of polymeric materials. Therefore it is worthily to investigate the effect of derived peptides addition to oil on the polymer content of sunflower oil used in frying

Table(4) shows the changes in polymer content of sun flower oil used for frying in the incorporated with antioxidant materials used in this study namely B1and B2 in comparison with control. It is well known that, sun flower oil used in the experiment has an initial polymer compounds content of less than 0.5% in case of standard sun flower and increase to about 1% on day 6 of frying IUPAC (1979) and Baileys (1996)

Table (4): Polymer content (%) in Frying Performance of Sunflower Oil during Batch Frying Potato in the presence of isolated peptides.

Groups	Time intervals (days)		
	0	3	6
Control	0.23	0.7	1
B1	0.2	0.35	0.75
B2	0.25	0.37	0.7

It could be seen from this table that gradually increase for control in polymers content were found to be 0.32, 0.7 and 1% at 0 time , after 3 days and 6 days respectively. The addition of B1 to the oil understudy caused a decrease in polymer content being 0.5 and 0.75% after 3 and 6 days respectively. Concerning B2 a decrease was recorded, being 0.6 and 0.81% after 3 days respectively.

Generally, the increase of polymers was due to the formation of high molecular weight namely polymerization. The addition of B1and B2 caused a decrease in polymer of about 25% and 19% after 6 days corresponding to control. The preceding data observed that the addition of derived peptides reduced the effect of frying temperature proceeds at interval time on oxidation of sunflower oil.

Considering the stability of corn oil and butter oil incorporated with derived peptides, the oxidative stability, or storage life until development of rancidity, which is an important factor in processing and marketing of fats, oils and fat containing foods.To achieve this purpose, rancimate method has been carried out for the measurement of the activity peptide incorporated with corn oil and butter oil against rancidity.

Table (5) illustrates the rancimat of control, BHA, peptide (1) and peptide (2) respectively. The obtained data of tested samples in comparison with the control revealed that incorporation of peptides (1 and 2) with corn oil caused an increase of stability period up to 19 hours for peptide (1) in comparison with control being 13.5 hours. Concerning peptide (2) a mild increase in comparison with control was found to be 15.6 hours

It can be concluded from the aforementioned data that peptide (1) was more effective on the stability of oils than incorporated one with BHA which were found to be 16.6 hours. This finding improved that, both peptides 1 and 2 were of special interest because of their relation with the stability of fatty foods .

Table (5): Oxidation stability of corn oil and butter treated with derived peptides and synthetic antioxidant (BHA) by Rancimat at 100°C.

Antioxidant	Induction period/h
	corn oil
control	13.5
BHA	16.6
B1	19
B 2	15.6

REFERENCES

Abd El-Khair, A. A., (2009). Formulation of milk permeate for utilization as Electrolyte Beverages. Australian J. Basic. Appl. Sci., 3(2): 572-578

A.O.A.C. (2007).Official methods of analysis of the association of official agricultural chemistis . 17th ed .

Baileys,(1996) industrial oil and fat products–Fifth Edition –Vol.1, John Wiley and Sons, New york. Chichester' Brisbane' Toronto.

Chan, K. M.and Decker, E. A. (1994). Endogenous skeletal muscle antioxidants. Crit. Rev.Food Sci. Nutr. 34, 403-426.

Chen, H. M., K, Muramoto, F. Yamauchi, K. Fujimoto and K. Nokihara(1998). Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein, J. Agric. Food Chem.46: 49-53.

Farag, R.S, El- Baroty .G.S, Basuny.A.M (2003). Safety evaluation of olive phenolic compounds as natural antioxidants. Int. J . Food Sci .Nutr. 54, 3 ,159- 174.

Francisco,J. H and Zamora,R. (2006). Peptides and proteins in edible oils : Stability, allergenicity, and new processing trends, Trends Food Science Technology.17 , 56-63.

Hernandez-Ledesma, B.; A. Davalos, B. Bartolome and L. Amigo,(2005) Preparation of antioxidant enzymatic hydrolyzates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS, J. Agric. Food Chem. 53, 588-593

IUPAC (1979). Standard methods for the analysis of oiles, fats and derivatives. Sixth ed., Pergamon Press, oxford. P. 96-108.

James A. Saunders, June M. Sanders, Samuel Morris and Spencer A. Wynne, II, (1988). Amino acid analysis of subcellular fractions by PITC and OPA, chromatogram. Volume 9, Number 1.

Korhonen,H.; and Pihlanto,A. (2006) Bioactive peptides Production and functionality. Int. Dairy J., 16, 945–960.

Lammli, U.K (1970): Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature 277:680-635.

Lopez –Exposito.I , Quires. A, amigo. L, and Recio.I (2007): casein hydrolysates as asource of antimicrobial , antioxidant and antihypertensive peptides, J.Dairy Science and Technology, 87. 4-5, 241-249.

Lottspeich, F. and H. Zorbas (1998): Bioanalytic Chap. 3, protein determination pp 42-45 Spectrum Acad. Verlag. Berlin Germany.

Mendez, E.;Sanhueza, J.; Speisky,H and Valenzuela, A.(1996) : Validation of the rancimat test for the assessment of the relative stability fish oils. J.Am. Oil Chem... Soc, 73, 8: 1033-1037.

Nielsen,S.;(1998) Food Analysis- Second Edition, Purdu University- West Lafayette, Indiane, Maryland.

Nogala-Kalucka.M,J., Korczak,M. Dratwia, E.Lampart-Szczapa, A.Siger and M.Buchowski (2005).Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chemistry, 93:227-235.

Park,E.Y , Nakamura.Y, Sato.K. and Matsumura,Y (2011). Effects of amino acids and peptide on lipid oxidation in emulsion systems. J. American Oil Chemists Society. 10:1940-7.

Pei- fen, W and Nawar ,W.W.(1986) : A technique for monitoring the quality of used frying oiles .J .Am .Oil Chem. Soc.,63, (10), 1363-1367.

Pearson,D. (1976). Chemical analysis of foods. Seventh. Edition. Churchll livingstone, Edinburgh London and New York.PP.

Rival, S.; Fornaroli, C., Boeri,G. and Wichers, H. J.(2001),Caseins and casein hydrolyzates. I. S. G. Lipooxygenase inhibitory properties, J. Agric. Food Chem., 49: 287-294

Sherwin,E..Jaocs, R, (1968), Evaluating antioxidant effectiveness in fats, oils, and fat-containing foods, J. AOCS . 45(11) : 632A, 634A, 646A,649A.

Suetsuna, K.,H. Ukeda and Ochi, H., (2000),Isolation and characterization of free radical scavenging activities peptides derived from casein, J. Nutr. Biochem., 11: 128-131.

Sultana. B, Anwar. F , Przybyiski. R (2007) . Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating . Food Chem. 104: 997-1005.

Uchida, K. and Kawakish, S. (1992). Sequence-dependent reactivity of histidine-containing peptides with copper (II)/ ascorbate. J. Agric. Biol. Chem... 40, 13-16.

Walker, J.M. (2002). SDS- polyacrylamide gel Electrophoresis of peptides .The protein Handbook, part 11, 3-79. Humana Press.

White, J.A, and Hart, R.J.C. (1986). An evaluation of the waters Pico-Tag System for the amino-acid analysis of food materials J. Automatic Chem. 8. 170-177.

البيتيدات المستخلصة من راشح اللبن فوائدها الصحية: صفاتها وتطبيقاتها العملية

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**** معهد الدراسات والبحوث البيئية**

***** الهيئة القومية للوقاية والبحوث الدوائية**

تهدف هذه الدراسة إلى عزل ببتيدات طبيعية ذات نشاط بيولوجى من هذا الراشح واختبار تأثيرها الايجابي ضد حدوث التزنخ التاكسدى لبعض الزيوت الغذائية مقارنة بمضاد اكسدة صناعى Butylated hydroxyanisol ولعزل هذه البتيدات تم استخدام الراشح المجفد بعد استرجاعه وتمت عملية إزالة المعادن باستخدام عمود سيفادكس G-10 ,وتم الفصل في مجال الهجرة الكهربائي باستخدام جهاز الالكتروفوريسيز. ولقد أظهرت النتائج المتحصل عليها بإجراء عملية الفصل على البولى اكريلاميد جيل بنسبة ١٢,٥ % فى الحصول على ببتيدين متقاربى الوزن الجزيئى حوالي ٢٠٠٠دالتون والآخر وزنه الجزيئى حوالي ٦٦٠٠ دالتون على التوالي والاختلاف في الوزن الجزيئى بين هذين البتيدين يعزى إلى اختلاف كل منهما عن الآخر في تركيز الأحماض الامينية وهى اسبارتيك . ثيريونين . ارجنين . فالين . ميثيونين . فينيل آلانين . هيستيدين . تربتوفان . ليسين – ولتقدير النشاط الكلى المضاد للأكسدة وجد أن كل ١٠٠ميكرو جرام من ببتيد ١وببتيد ٢يكافى ٩,٤٦ و٧,٩٤ منسوبا لفيتامين سي على التوالي وأظهرت التجارب الخاصة بثبات هذه البيتيدات عدم تأثير زمن عملية القلي عليها .كما أظهرت النتائج دور هذه البيتيدات المستخلصة في تقليل التزنخ التاكسدى لكل من زيت الذرة وزيت الزبد مقارنة ب BHA والكنترول المستخدم . وبخلط هذين البتيدين مع زيت عباد الشمس كان له دور فعال في تقليل محتوى الزيت من البوليمر بنسبة تراوحت ما بين ١٩% إلى ٢٥% بعد ٦ أيام مقارنة بالكنترول . إضافة إلى ذلك انه بإجراء اختبار الرانسيمات ثبت أن هذه البيتيدات المستخلصة لها تأثير على ثبات الزيت المستخدم مقارنة ب BHA . ولقد اثبتت الدراسة فعالية هذه البيتيدات كمضادات للتزنخ التاكسدى لبعض الزيوت وزيوت الزبد لذا فانه ينصح باستخدامها كمواد طبيعية تستخدم كمضادات للأكسدة في الأغذية والمكملات الغذائية .

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