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Effect of Trypsin on The Functional Properties of Salted and Ultrafiltrated Whey Protein Concentrates

Eman M. Ibrahim* and Mohamed Abed Ghanimah**

**Department of Dairy Science, Faculty of Agriculture, Kafrelsheikh University, Egypt, Prince Sattam Bin Abdulaziz University,Al Kharj, Saudi Arabia,*

***Department of Dairy Science, Faculty of Agriculture, Kafrelsheikh University, Egypt*

THIS RESEARCH aimed to study the effect of hydrolysis of whey protein concentrates (WPCs) using trypsin enzyme on their functional properties and use of the modified concentrates in the manufacture of yogurt. Two types of whey were used, sweet whey from Ras cheese and salted whey from Domiati cheese manufacture. Whey protein concentrates were prepared by ultrafiltration of the sweet whey (UFWPC), and precipitation of the salted whey (SWPC) at 90°C / 20 min and pH 4.6. The prepared whey protein concentrates were treated with trypsin enzyme at concentrations of 0.15, 0.3 and 0.5 g enzyme per 100 g protein, and freeze dried. The results showed that the hydrolysis of whey protein concentrates had insignificant effect on the moisture, fat, ash and lactose, while the total nitrogen content and the degree of degradation significantly increased by increasing the concentration of the enzyme. Water and fat absorption capacities were increased by increasing the enzyme concentration and were higher in UFWPC. Emulsification capacity, foaming properties and the ability to form gel were improved by increasing the concentration of the enzyme. The replacement of skim milk powder with hydrolyzed WPCs (by 0.5 g trypsin /100 g protein) in the manufacture of yogurt increased TS, TN and ash content and decreased the pH . Curd syneresis significantly decreased in all treatments compared to control, however treatments with UFWPC exhibited the lowest values. The substitution of 50% and 75 % skim milk powder (SMP) with SWPC and UFWPC in order, had no effect on sensory properties of the yogurt compared to control.

Keywords: Trypsin, Whey protein concentrates, Solubility, Emulsification, Foaming, Yogurt, Syneresis

Introduction

Whey, the important by-product of the cheese industry, contains highly nutritional and biological proteins. Whey protein is an excellent source of essential amino acids needed by the human body, and branch chained amino acids (Jimenez et al., 2012 and Yadav et al., 2015). Whey protein products are considered as functional ingredients, and their functionality is related to the protein content and composition (Huck-Iriart et al.,2014 and Ghanimah, 2018).

Several factors could affect or alter the functional properties of whey proteins like the processing conditions. Sever heat treatment can impair the functional properties of whey proteins due to the denaturation and aggregation (Morr and Ha, 1993). The functionality of whey proteins can be improved by modification of protein via physical treatments and enzymatic modification (Kilara and Vaghela, 2018). It has been found that the functional properties can be improved by enzymatic hydrolysis of heat denatured whey protein (Kebary et al., 2009a). The modified whey proteins were more suitable for the use in the processed food industry, as well as they have health benefits, like antihypertensive, antibacterial, mineral-binding, antithrombotic and anti –gastric activities. (Gowda et al., 2006, Mota et al., 2006, Prieto et al., 2014 and Jeewanthi et al., 2015 a,b).

Several enzymes were used in the food industry to produce whey protein hydrolysates (WPHs) with various functional properties and biological activity. The most used enzymes to produce WPHs are digestive enzymes like trypsin, pepsin, and chymotrypsin. (Jeewanthi et al., 2015 a,b).

*Corresponding author : Email: e_ibrahim1972@yahoo.com

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Yogurt is an important dairy product in the world. The fortification of yogurt milk with skim milk powder improves the quality of the yogurt and prevents syneresis (Karam et al., 2013).

The present study was undertaken to study the effect of characterize hydrolysis of whey protein concentrates (WPCs) using trypsin and use of the modified concentrates in yogurt manufacture.

Materials and Methods

Materials

Trypsin 2000 used in the current study was obtained from Sigma Chemical Company St Louis, Mo, USA. Sweet whey from Ras Cheese manufacture was obtained from Mahmoud Mahdya Factory, Gharbia Governorate, Egypt. Whey was strained, separated using a cream separator to remove residual lipids, and then pasteurized. Whey was ultrafiltered using DDS Lap 20 UF equipment (APV/ Pasilac A / S, Silkeborg, Denmark; surface area 2 m2, GR 61 polysulphone membrane, Mo cut-off 20,000) at 40°C to a concentration of 28%. This concentrate was designated as ultrafiltrated whey protein concentrate (UFWP). Salted whey of Domiati cheese manufactured from buffalo milk was obtained from a local factory. Whey protein concentrate (SWPC) was prepared by precipitation the salted whey at 90°C / 20 min., and pH 4.6.

Condition of the enzymatic hydrolysis of whey protein concentrates

The hydrolysis of whey protein concentrates with trypsin enzyme at concentrations of 0.15(T1), 0.3(T2) and 0.5(T3) g enzyme per 100 g protein was carried out as described by Kebary et al. (2009a). The pH was adjusted to 7.6 for the action of trypsin enzyme and incubated at 25°C for 2h, and to stop the enzyme action the temperature was raised to 75°C for 10 min. The hydrolyzed whey protein concentrates were frozen overnight and freeze dried at -60°C (Labconco Freeze Dryer 64312, Kansas, Missouri, USA.). The modified whey protein concentrates were analyzed for chemical composition and functional properties.

Methods

Chemical analysis

The degree of hydrolysis was determined as given by Yamashita *et al.* (1970). Moisture, fat, and ash contents were determined according to the methods given by AOAC (2000). The micro-Kjeldhal method (Ling, 1963) was followed for determination of NPN and TN, whereas protein content was calculated from the following equation: Protein = TN×6.38. Lactose content was determined according to the method of Barnett and Abd El-Tawab (1957).

Functional properties:

The solubility was determined according to Smith et al. (1959). Water absorption was measured according to the procedure of Sosulski (1962). Fat absorption was determined according to Sosulski et al. (1976). Emulsification capacity was determined according to Beuchat et al. (1975). The method of Lawhon et al. (1972) was used to determine foaming capacity(%) and foam stability. Gelation was measured according to the method described by Utsumi and Kinsella (1985).

Preparation of yogurt

Fresh cow milk (3% fat and 8.5% SNF) was supplemented with SMP (3%) and served as a control. The skim milk powder was replaced with 50%, 75% and 100% hydrolyzed SWPC (YS1), (YS2) and (YS3), or hydrolyzed UFWPC (YUF1), (YUF2) and (YUF3). Yogurt was manufactured according to Tamime and Robinson (1999).

Chemical analysis of yogurt

Fresh and stored yogurt (for 12 days in a refrigerator) samples were analysed for total solids, ash and pH according to the methods given by AOAC (2000), TN was determined according to Ling (1963), and the protein content was calculated from the following equation: Protein = TN×6.38.Curd syneresis was determined according to Mehanna and Mehanna (1989). Sensory evaluation was determined according to Narayana and Gupta (2013).

Statistical analysis

The ANOVA and Duncan's test (at $P<0.05$) were carried out and the average and standard error were calculated using the SPSS program (version 16), SPSS Inc., Chicago, IL, USA.

Results and Discussions

Table 1 shows that the addition of trypsin to the two types of whey protein concentrates gradually and significantly increased the degree of hydrolysis. However, the degree of hydrolysis was significantly higher in UFWPC than that in SWPC. Similar trends were observed by Jeewanthi et al. (2015b).

Table 2 shows the effect of the trypsin enzyme concentration on the chemical composition of whey protein concentrates. There were no significant changes in the moisture, lactose, fat, and ash, while there were significant differences in the total protein and NPN in both whey proteins concentrates. These results agree with Jeewanthi et al. (2015b).

Functional properties

The solubility significantly increased by increasing the concentration of enzyme used. The modified and unmodified UFWPC exhibited much higher solubility indexes than those of SWPC (Table 3). Limited hydrolysis of protein leads to the reduction in the molecular weight and the increase in the solubility and the hydrophilicity resulting from the increase in amine groups and free carboxyl (Jeewanthi et al., 2015a). More extensive hydrolysis has been shown to increase the solubility (Flanagan and Fitzgerald, 2002). Jeewanthi et al. (2015 b) found that the solubility of WPHs was higher than that of untreated WPCs, and they attributed that to the increase in the number of ionizing groups (NH4+, COO-) and hydrophilicity with enzymatic hydrolysis.

It is clear from Table 3 that water and fat absorption capacities significantly increased by increasing the concentration of trypsin. Both modified and unmodified UFWPC exhibited higher water and fat absorption capacities than those of SWPC. Increased fat binding capacity was associated with an increase in hydrophobicity of the protein (Voutsinas & Nakai, 1983 and Jeewanthi et al., 2015 a). Modification of SWPC and UFWPC with trypsin caused a significant increase in their emulsification capacities and this improvement increased by increasing the concentration of enzyme. The emulsification capacities of UFWPC were higher than those of SWPC (Table 3). Limited enzymatic hydrolysis has been found to successfully improve the interfacial properties of WPCs (Foegeding et al. 2002, Kilara & Panyam, 2003 and Jeewanthi et al., 2015 a).

The results of foam capacity and foam stability are shown in Tables 3 and 4 , respectively. It is clear that foam capacity and foam stability increased by increasing the trypsin concentration in all treatments. Limited enzymatic hydrolysis promotes foaming aeration through more rapid absorption at the interface by reducing the peptide size (van der Ven et al., 2002). Proteins stabilize foam by strongly adsorbing to the air-water interfaces, forming viscoelastic adsorbed layers and leading to protein network with high viscosity (Rullier et al., 2010).

A gel is an intermediate structure between solid and liquid, which protein strands crosslink to form a network. Gelation is favored by large molecules of proteins, which form extensive networks by cross-linking in three dimensions and the ability of the denaturing (Jeewanthi et al., 2015 a). The ability of the protein to form gel increased by increasing the trypsin concentration in all treatments (Table 3).

In general, the hydrolysis of proteins by proteolytic enzymes leads to a reduction in the protein molecular weight, an increase in the ionizable groups, and the exposure of hydrophobic groups. These changes had a direct effect on the functional properties of the protein (Kilara and Vaghela, 2018).

TABLE 1. Effect of adding Trypsin to whey protein concentrates on the degree of hydrolysis (%) (Data are means ± SE)

Type of WPC	Control	Treatments (degree of hydrolysis%)		
		T1	T2	T3
SWPC	0	3.0^{cB}	4.8^{bB}	6.5^{aB}
UFWPC	0	4.2^{cA}	7.6^{bA}	9.3^{aA}

T1, T2 and T3: whey protein concentrates hydrolyzed with trypsin at the rate of 0.15, 0.3 and 0.5 % of protein respectively. SWPC: Salted Whey Protein Concentrate, UFWPC: Ultrafiltrated Whey Protein Concentrate.

Means in the same raw(treatments) with different lowercase superscripts are significantly different ($P < 0.05$).Means in the same column(type of WPC) with different uppercase superscripts are significantly different ($P < 0.05$).

TABLE 2. Effect of adding Trypsin on the chemical composition of SWPC and UFWPC (Data are means ± SE)

Chemical Composition	SWPC				UFWPC			
	Control	T1	T2	T3	Control	T1	T2	T3
% Moisture	6.04^{a}	6.07^{a}	6.11^{a}	6.15^{a}	7.10^{a}	7.10^{a}	7.16^{a}	7.19^{a}
Lactose%	13.0^{a}	13.06^{a}	13.08^{a}	13.10^{a}	16.70^{a}	16.82^{a}	16.75^{a}	16.78 ^{a}
Fat%	1.1^{a}	1.1^{a}	1.1^{a}	1.1^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
Total Protein%	67.30^{d}	68.01^{c}	69.07^{b}	70.0^{a}	61.20^{d}	61.80^{c}	62.20^{b}	62.88^{a}
Total ash%	9.00^{a}	9.05^{a}	9.07^{a}	9.22^{a}	10.60^{a}	10.63^{a}	10.65^{a}	10.72^{a}
NPN%	0.73^{d}	1.36^{c}	1.89^{b}	2.20^{a}	0.90^{d}	1.77^{c}	2.28^{b}	2.83^{a}

See Legend to Table 1 for details

Means in the same row with different lowercase superscripts are significantly different ($P < 0.05$).

TABLE 3. Effect of adding Trypsin on functional properties of SWPC and UFWPC (Data are means ± SE)

Functional properties	SWPC				UFWPC			
	Control	T1	T2	T3	Control	T1	T2	T3
Protein solubility index	17.55^{c}	32.50^{b}	45.10^{a}	46.22^{a}	23.0^{c}	23.00^{c}	90.85^{b}	92.14^{a}
Water absorption capacity (g/g)	2.31^{d}	3.73^{c}	4.22^{b}	4.91^{a}	2.54^{d}	4.27^{c}	4.77^{b}	5.38^{a}
Fat absorption capacity (g/g)	1.52^{c}	2.03^{b}	2.36^{a}	2.42^{a}	1.77^{d}	2.42^{c}	2.75^{b}	3.17^{a}
Emulsification capacity (ml of oil/g of sample)	201.4^{d}	220.50^{c}	235.20^{b}	245.3^{a}	211.3^{d}	231.52^{c}	260.91^{b}	266.3^{a}
Foam capacity (%)	9^{c}	25^{b}	25^{b}	28 ^{a}	10^{d}	27^{c}	32^{b}	35^{a}
Gelation Capacity (%)	18.5^{a}	16.5^{b}	14.50^{c}	11.5^{d}	17.5^{a}	15.5^{b}	14.1^{c}	11.2^{d}

See Legend to Table (1) for details

Means in the same row with different lowercase superscripts are significantly different ($P < 0.05$)

TABLE 4. Effect of adding Trypsin on foam stability of SWPC and UFWPC (Data are means ± SE)

Standing Time (min)	SWPC				UFWPC			
	Control	T1	T2	T3	Control	T1	T2	T3
0	18^{A}	38^{A}	50^{A}	55^{A}	36^{A}	48 ^{A}	57 ^{A}	66^{A}
15	15^{B}	21^{B}	30^{B}	35^{B}	34^{B}	42 ^{B}	47^{B}	54^{B}
30	13^{C}	18^{C}	21^{C}	25^{C}	32^{C}	37^{C}	40^{C}	42^{C}
45	12^{C}	14^{D}	15^{D}	17^{D}	30^{D}	34 ^{D}	36^{D}	38^{D}
60	11^{C}	13^{D}	14^{D}	15^{E}	25^{E}	32 ^{E}	34^{E}	35^{E}
75	10^{D}	9^{E}	9^{E}	9^{F}	25^{E}	22 ^{F}	24^{F}	25^{F}
90	10^{D}	6^{F}	7^{F}	8^{F}	25^{E}	21^{F}	24^{F}	24^{F}

See Legend to Table (1) for details

Means in the same column with different uppercase superscripts are significantly different ($P < 0.05$)

Chemical composition and sensory properties of yogurt

It was noticed from Fig. 1 that replacement of SMP with the modified WPCs (using 0.5 g trypsin / 100 g protein) increased the TS, protein and ash content and decreased the pH value compared with control, while the type of WPC had insignificant effect. These results agree with Kebary et al. (2009 b) and Roumanas et al. (2016). Figure 2 shows that the replacement of SMP with modified WPCs significantly decreased ($P<0.05$) the curd syneresis compared with control, and the yogurt with UFWPC had the lowest values. These results may be due to the high protein content in treated yogurt samples. Curd syneresis decreased after 6 days and increased at the end of the storage (12 days). The sensory properties improved (Fig. 3), and the substitution of SMP with SWPC and UFWPC up to 50% and 75%, in order, did not affect the sensory properties of the treated yogurt samples compared to the control.

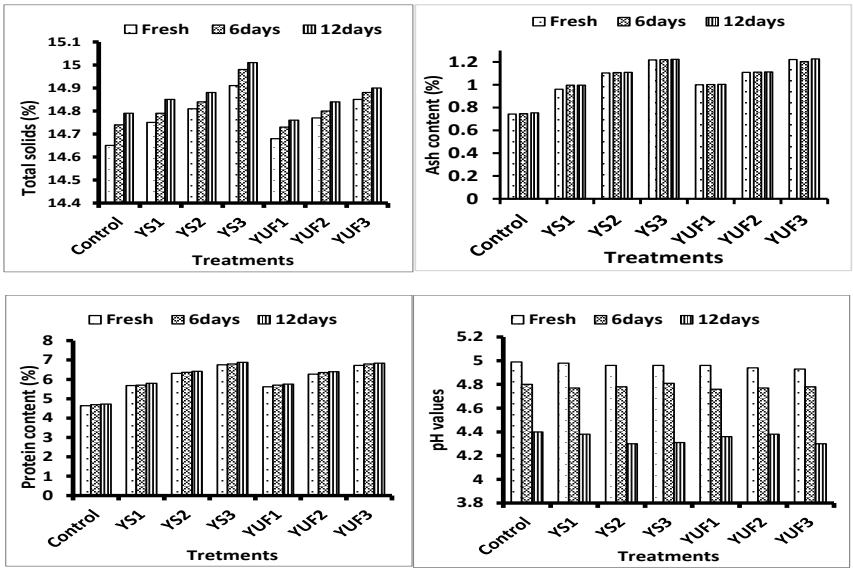

Fig 1. Effect of replacement of skim milk powder with modified whey protein concentrates on some chemical properties of yogurt

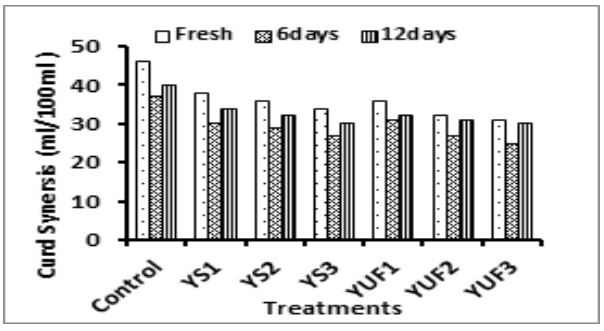

Fig 2. Effect of replacement of skim milk powder with modified whey protein concentrate on curd syneresis of yogurt

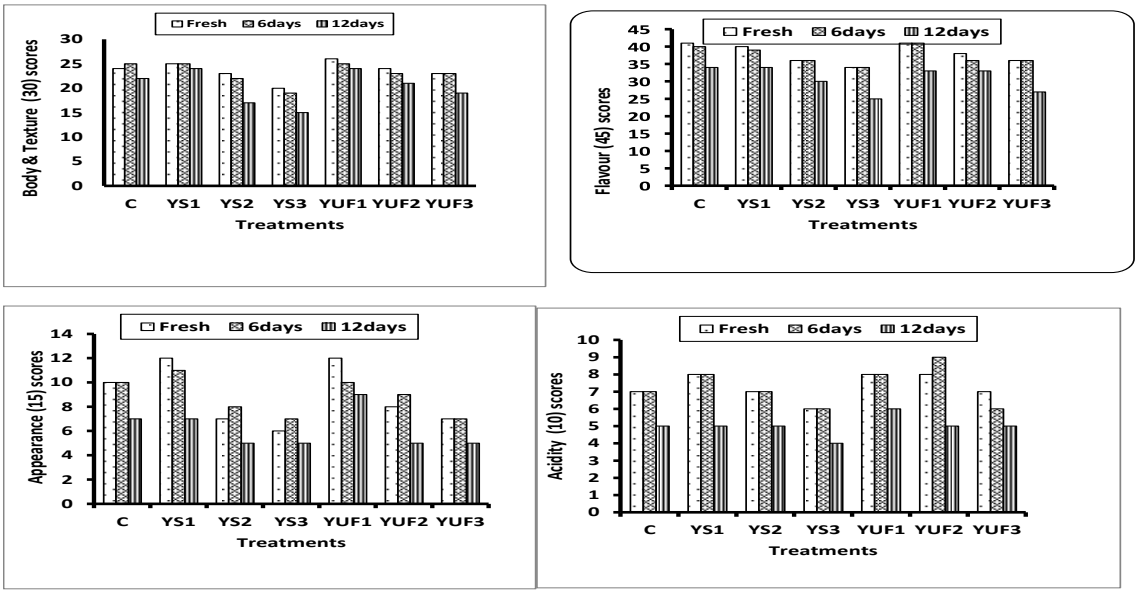

Fig 3. Evaluation of sensory properties of yogurt made with hydrolyzed whey protein concentrates during storage period

Conclusions

It could be concluded that the modification of the prepared WPCs using trypsin significantly improved their functional properties. The modified whey protein concentrates with 0.5 g trypsin /100 g protein can be used to replace skim milk powder to enhance the properties of Yogurt.

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تأثير التربسين على الخواص الوظيفية لمركزات بروتين االشرش المملح و الشرش المركز بالترشيح الفائق

إيمان ابراهيم و محمد عابد غنيمه
كلية الزراعة - جامعة كفر الشيخ - كفر الشيخ - مصر

يهدف هذا البحث الى دراسة تأثير تحلل مركزات بروتين الشرش باستخدام تركيزات مختلفة من انزيم التربسين على خواصها الوظيفية واستخدام المركزات التي سبق تحليلها في صناعه اليوغورت. تم في هذه الدراسة استخدام نوعين من الشرش وهو شرش حلو ناتج من صناعه الجبن الراس وشرش مملح ناتج من صناعه الجبن الدمياطي. تم تحضير مركزات بروتين الشرش بإجراء الترشيح الفائق للشرش الحلو بينما تم استخدام الترسيب للشرش المملح على ٩٠°م لمده ٢٠ ق مع ضبط ال pH عند ٤,٦ لتحضير مركز بروتين الشرش المملح . تم معامله كلا النوعين من المركزات المحضرة بإنزيم التربسين باستخدام تركيزات ٠,١٥،٠,٣،٠,٥ جم انزيم/ ١٠٠ جم بروتين ثم تم تجفيد المركزات. اشارت النتائج ان تركيز انزيم التربسين المستخدم لم يكن له تأثير معنوي على المحتوى من الرطوبة ، الدهن ،الرماد واللاكتوز بينما زاد المحتوى من النيتروجين الكلى ودرجه التحلل بصوره معنويه بزيادة تركيز الانزيم المستخدم. زادت القدرة علي ربط الماء والدهن بصوره معنويه بزياده تركيز الانزيم وتميز المركز الناتج من الترشيح الفائق بأعلى القيم . تحسن الاستحلاب وخواص تكوين الرغوة والقدرة على تكوين الجل بزياده تركيز التربسين المستخدم. أدى استبدال اللبن الفرز المجفف في صناعه اليوغورت بمركزات بروتين الشرش التي سبق تحليلها باستخدام ٠,٥ جم انزيم تربسين /١٠٠ جم بروتين الى زياده الجوامد الصلبة الكلية و النيتروجين الكلى والرماد بينما انخفض ال pH . انخفض التشريش معنويا في كل المعاملات مقارنه بعينه المقارنة الا ان المعاملات المصنعة باستخدام المركز الناتج بالترشيح الفائق أظهرت اقل قيم تشريش. لم يؤثر استبدال اللبن الفرز المجفف بمركزات الشرش المحللة إنزيميا والناتجة من الشرش المملح علي خواص الزبادي الناتج حتي نسب استبدال ٥٠٪ ، كذلك لم يؤثر الاستبدال حتي ٧٥٪ في حالة المركز المحللة انزيميا الناتج من الترشيح الفوقي علي خواص الزبادي الناتج .