

Production of infant food fortified with partially hydrolyzed wheat protein

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ABSTRACT

Improvement of functional properties of infant cereal protein by hydrolyses of wheat protein using papain enzyme compared with adding papaya pulp as source of the papain enzyme were studied. Studies indicated that the degree of hydrolysis (DH) was increasing by increasing the time of incubation till 60 min. the (DH) was reach the maximum hydrolysis percentage and record 2, 14, 16% for control, papain enzyme and papaya pulp respectively. The obtained result show that the infant cereal produces by adding both papain enzyme and papaya pulp was best in its nutrient values, protein efficiency ratio C-PER and quality compared with control. Added the papaya pulp increase the nutritional value of infant cereals due to the high amount of nutrients that papaya have such as beta carotene, vitamin C and other vitamins and minerals.

Keywords: *wheat, papaya, protein hydrolysis, papain enzyme, drum drier, infant food.*

INTRODUCTION

To improvement of functional properties of protein hydrolysates, some studies dealt with the production of low molecular peptides. These peptides have the advantage of being absorbed in the intestine without any digestion in the stomach and have low allergenic effects (Schwartz *et al.* 2011 and Gonzalez-Tello *et al.* 1994). This explains their preferential use in many formulas such as diets for nursing infants or sick adults and as stimulations for persons liable to develop allergy.

Pe'rez-Conesa *et al.*, (2002) conclude that the heat processing and enzymatic treatment of the infant cereals tested, significantly affected the protein content either crude or true protein content, especially in gluten-free cereals blend (Rice and carrot), which showed lower values of all parameters studied than infant cereals containing gluten (Multi cereals and Wheat). However, in vitro protein digestibility showed significant differences during

processing, the values remaining high in all the samples from raw to commercial product. The calculated protein efficiency ratio C-PER increased significantly as a result of technological treatment in all the infant formulas due to the high values of in vitro protein digestibility and essential amino acids used to determine the protein quality parameter, although there were no significant differences between the four infant cereals. Therefore, it can safely be said that the infant cereals analyzed supply the necessary amount of essential amino acids for infant.

Xiangzhen *et al.*, (2006) examine the use of commercially available proteolytic enzymes for the preparation of wheat gluten hydrolysates (WGHs), to characterize the hydrolysates by determining their solubility. Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates by controlling the reaction conditions during the enzymatic hydrolysis, it is

possible to obtain hydrolyses having different characteristics.

Much research focused on chemical or enzymatic modifications of wheat gluten had resulted in the enhancement of its solubility, foaming and emulsifying properties (**Batey 1985; Kammoun *et al.*, 2003; Linare`s *et al.*, 2000; Mimouni *et al.*, 1999 and Popineau *et al.*, 2002**). Different proteases have been tried, such as pepsin, trypsin, papain, bromelain. Most work on enzymatic hydrolysis of wheat gluten focused on peptic hydrolysis, which is known to have a broad side chain specificity and to produce large polypeptides from gluten (**Cornell and Mothes 1995 and Masson *et al.*, 1986**). Wheat gluten hydrolysis results in peptide mixtures with high solubilities and altered foaming and emulsifying characteristics, depending on the degree of hydrolysis.

The main objective of the present investigation is to study the use of commercially available papain enzymes

comparing with adding papaya pulp for the preparation of wheat based infant cereals with wheat protein hydrolytes (WPHs).

MATERIALS AND METHODS

Materials:

The raw materials for infant cereal formula skimmed milk, sugar, wheat, vitamin and minerals were obtained from the Arab Company for Medicinal Food (Medi Food) Abo sultan Ismailia, Egypt. Fresh ripe papaya fruits were purchased from the local market. The papain enzyme was purchased from Infoenzyme Company, Germany, with specification 2 million units per gram.

Methods:

Preparation of drum-dried infant-cereal with enzyme and papaya pulp:

Infant cereal formula was prepared using the master formula produced by the Arab Company for Medicinal food, Ismailia, Egypt, as shown in Fig.(1) with modification by adding papain enzyme 3g/kg formula (0.3%) and papaya pulp 50g/kg formula (5%). All trails

were incubated at 42°C for different times 0-90 min.

The ripe papaya fruits were washed, peeled, cut into pieces and pulped to produce papaya pulp.

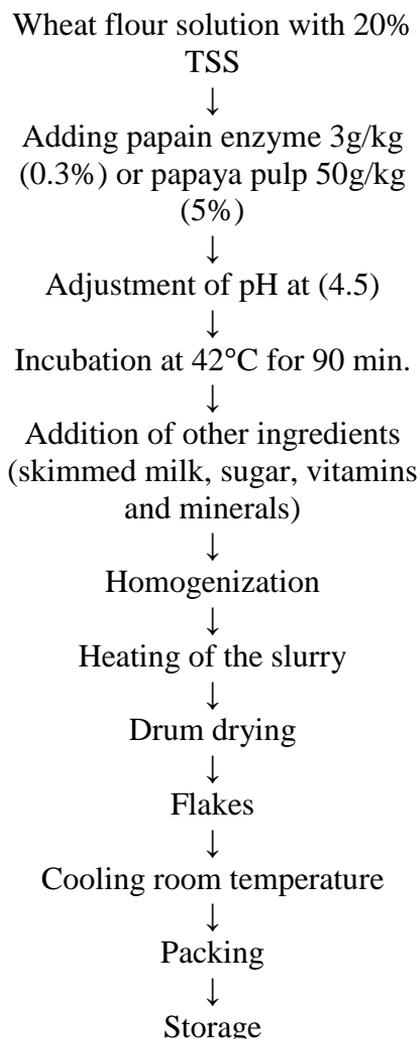


Fig. (1): General processing steps for cereal flakes with papaya treatment.

Physical and chemical characteristics determination for cereal flakes.

Total soluble solids (°Brix), viscosity, non-enzymatic browning, pH, acidity (as % anhydrous citric acid), total carotenoids and moisture of cereal flakes formulas were determined according to **Sharma et al (2013)**. Fat and protein were determined according to **(A.O.A.C, 2005)**. Carbohydrates were calculated by difference

Determination of cereal flakes

Minerals:

Ash content was measured by calcinations, overnight at 550°C in a furnace, to constant mass **(A.O.A.C, 2005)**. Sodium, potassium, magnesium, manganese, iron, calcium, chloride, copper, iodine, phosphorus and zinc were determined using atomic absorption spectrophotometer (Perkin Elmer model 3300, Merck hydride system USA) according to the method of **(A.O.A.C, 2005)**.

Determination of cereal flakes vitamins:

Vitamin A (retinol), vitamin B group (B1, B2...), vitamin C and vitamin (D and E) were determined by HPLC according to the method described by **Leth and Jacobsen, (1993); Bogner (1992); Romeu- Nadal et al. (2006) and A.O.A.C (2005)**, respectively.

Determination of cereal flakes color:

The color was determined at three different points of the sample with a Chroma meter (Minolta CR 400, Minolta Camera, Co., Osaka, Japan) equipped with an 8 mm measuring head and a D65 illuminant. Color changes were quantified in the L*, a*, b* color space. The calorimeter was calibrated with a standard white ceramic plate (L = 95.97, a = - 0.13, b = - 0.30) prior to reading. Corresponding L* value (lightness of color from zero (black) to 100 (white); a* value (degree of redness (0–60) or greenness (0 to - 60); and b* values (yellowness (0–60) or

blueness (0 to - 60) were measured for all the samples. Colors were calculated according to method of **Abonyi et al., (2002)**.

Determination of cereal flakes total and reducing sugars:

The total sugars of the various fractions of the samples were estimated as per the method of Lane-Eynon (**Chris et al., 1998**), which comprised of 10 g sample mixed with 10 ml of concentrated HCl and kept overnight for inversion of sugars. The inverted sugar samples were made up to volume (100 ml) after adding few drops of phenolphthalein indicator and neutralized with 1 N NaOH till pink color appears. The inverted sugar sample titration was done against Fehling's A and B solutions (10 ml each) with 10 ml of distilled water. The methylene blue was used as an internal indicator and the end point was noted after getting the brick red color. Similarly, the reducing sugars of the samples were analyzed without inverting the sugars. The

volume was noted and calculated as per the following formula:

$\% \text{Total sugars} = \text{Fehling's factor} \times \text{Dilution} \times 100 / \text{Titre value} \times \text{weight of the sample.}$

The same procedure was followed for reducing sugars without inverting the sugars.

Microbiological evaluation of cereal flakes:

The microbiological methods described by Merck (1977) were used for detecting the safety value of cereal flakes formula.

Determination of the degree of hydrolysis (DH) using Trichloroacetic acid (TCA):

Peptic hydrolysis, DH was determined by the ratio of the percentage of 10% TCA-soluble nitrogen to total nitrogen in the sample. Aliquots were removed at the final time required and mixed with 20 % TCA to create 10% TCA-soluble and TCA-insoluble fractions. After 30 min, the mixture was centrifuged at 3000 rpm and the supernatants were analyzed for nitrogen by the semi micro-

Kjeldahl method A.O.A.C. (2005).

Calculation of protein efficiency ratio C-PER for cereal flakes:

The Calculated of Protein Efficiency Ratio (C-PER) has been proposed as a rapid assay, which combines data from in vitro protein digestibility (IVPD) and amino acid composition to predict protein quality. In this study C-PER was calculated according to the method of (Satterlee et al., 1982).

Sensory evaluation cereal flakes:

The sensory quality of infant cereal flacks from this process was evaluated by 10 mothers infant who evaluated the product for color, flavor, taste, odor, rehydration and overall acceptability according to (Sharma et al., 2013).

Statistical analysis:

Statistical analysis were carried out using single analysis of variance (ANOVA) significant level of $p < 0.05$ in

Microsoft Excel Software-2000 of Windows-XP. (Juan et al. 2015)

RESULTS and DISCUSSION

In this study papain enzymes and papaya pulp were used to partially hydrolyzed wheat protein to be healthier for infant. During preparing the papaya pulp it was found that the peeling losses and yield of pulp were 32 and 66% respectively **Ezekiel and Florence (2012)**. The operational data of drum dried cereals flakes were present table (1) the data showed drum drier operational parameters were drum speed 10 ± 0.01 (rpm), steam pressure in heated drums 3.00 ± 0.02 (psi), clearance between the drums 0.55 ± 0.00 (mm), drying time to get drum-dried sheet 1.00 ± 0.01 min, thickness of the cereal flakes with papaya 1.00 ± 0.00 (mm) and yield of cereal flakes with papaya amounted is $76.0 \pm 0.0676\%$.

Enzymatic hydrolysis of wheat protein:

The control of enzymatic hydrolysis was related to the mechanism of proteolytic reactions involving a soluble enzyme and an insoluble substrate wheat protein **Nouri et al., (1997)**. The hydrolysis of wheat protein with papain enzyme, papaya pure and control were determined. (Fig. 2), indicating that degree of hydrolysis (DH) was increasing by increasing the time of incubation and after 60 min. of incubation the (DH) was reach the maximum hydrolysis percent and record 2, 16, 14 % for control, papain enzyme and papaya pulp respectively.

Calculation of protein efficiency ratio C-PER:

The protein efficiency ratio (PER) is recognized as the preferred parameter for reacting protein quality in clinical test. However, PER may overestimate the ability of animal protein to encourage growth, and underestimate ability of vegetable protein. Figure 3 presents the trend shown by C-PER, which is used to estimate protein quality as a

combination of digestibility and the essential amino acid score during processing. In general, C-PER increased significantly ($P < 0.001$) with processing, providing values in the final product very close to 3.2 in both infant cereal with papain enzyme and papaya pulp while it was 2.3 in control. The values observed were similar to those reported by **Pe'rez-Conesa et al., (2002)** and **Ezekiel and Florence (2012)**.

Physicochemical characteristics of ripe papaya fruits:

Data in Table (2) show the physicochemical characteristics of ripe papaya fruit moisture, total sugar, reducing sugar, vitamin C (mg/100g) and total carotenoids were (87 ± 0.08 , 8.69 ± 0.06 , $6.40 \pm 0.04\%$, 46.55 ± 0.4 and 4.29 ± 0.2 mg/100g) respectively, this result indicate that papaya fruit is good source of some important nutrients such as vitamin C and beta-carotene which conceder as pro vitamin A (**Ezekiel and Florence 2012**). The color values L, a and b of ripe papaya pure were 37.51, 9.15 and 23.47 respectively,

from this result it could be notice that increase the b value indicating the yellow color of papaya pure.

Sensory evaluation of cereal flakes formulas:

The cereal flakes formulas (control, prepared with papain enzyme and papaya pulp) were organoleptic ally evaluated to assess the mothers infant's acceptability of these products (**Juan et al., 2015**). The samples were evaluated for color, taste, odor, flavor, rehydration and overall acceptability. Sensory evaluation data were statistically analyzed and the results obtained are given in Table (3). From table it could be notice that the sensory evaluation values of cereal flakes with papaya pulp was higher than control and infant cereal with enzyme and it is categorized very good. There were no significant differences between control and cereal flakes with papain enzyme only.

Physicochemical characteristics of infant-cereal with papaya pulp:

Physicochemical characteristics of infant cereal with papaya pulp were determined and the data showed in Table (4). From the table observe that total acidity, fat, vitamin C and total carotenoids were high in infant cereal prepared with 5% papaya pulp than control (0.54, 6.28%, 41.6 mg/100g and 7.34 mg/100g respectively). For the color value results notice that the L value for infant cereal with papaya pulp was decreased comparing with control infant cereals (54.73 and 73.3 respectively) while a and b values were increased from 6.55 and 18.41 in control to 8.11 and 30.30 in infant cereal with papaya pulp respectively. It mean that the product with papaya become more yellow than control and high in nutrients by adding papaya pulp (Jeana et al 2013). For protein and reducing sugar value were decreased from 15 and 58.26 to be 14.63 and 56.78 % respectively.

Vitamins and minerals composition of infant cereals with papaya pulp:

Vitamins and minerals contents of infant cereals with papaya pulp are show in Table (5) result show that by adding papaya pulp to infant cereal formula makes the product high in minerals and vitamins content comparing to the control especially in Vitamin A and vitamin C as well as other nutrients. The infant cereals with papaya pulp covered high percentage of the RDA for vitamin (A, D3, E, B3, B12 and C) and minerals (calcium, iron, phosphorous, potassium, chloride, sodium, zinc, magnesium, iodine, copper and manganese) for infants (WHO 2011).

Microbiological results for cereal flakes formulas:

The microbiological count of cereal flakes formula prepared control, with Papain enzyme and with papaya pulp were determined and the data are showed in Table (6) the data indicate that the microbial count were confirmed with the microbiological standard for infant cereal products (WHO

2011) and free of *E. coli*,
Salmonella and Staphylococcus.

CONCLUSION

It could, concluded that to improvement functional properties of infant cereal flakes protein by using papain enzyme compared with added papaya pulp as source of the papain enzyme to hydrolyze wheat protein. Studies indicated that the degree of hydrolysis (DH) was increasing by increasing the time of incubation till 60 min. the (DH) was reach the maximum hydrolysis percentage when adding papain enzyme or papaya pulp. The infant cereal produced by adding papaya pulp was best in its nutrient values, protein efficiency ratio C-PER and quality than control.

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Table (1): Operational data of drum dried cereal flakes with papaya pulp

Parameters	Values
Drum speed (rpm)	10±0.01
Steam pressure in heated drums (psi)	3.00±0.02
Clearance between the drums (mm)	0.55±0.0
Drying time to get drum-dried sheet (min)	1.00±0.01
Thickness of the infant cereal flakes with papaya (mm)	1.00±0.0
Yield of cereal flakes with papaya (%)	76.0±0.06

*Means of triplicate ±SD.

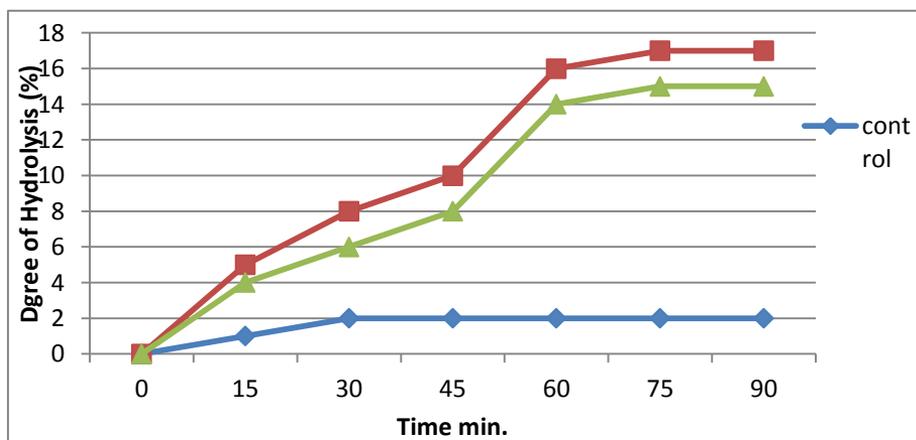


Fig. (2): Enzymatic hydrolysis of wheat protein

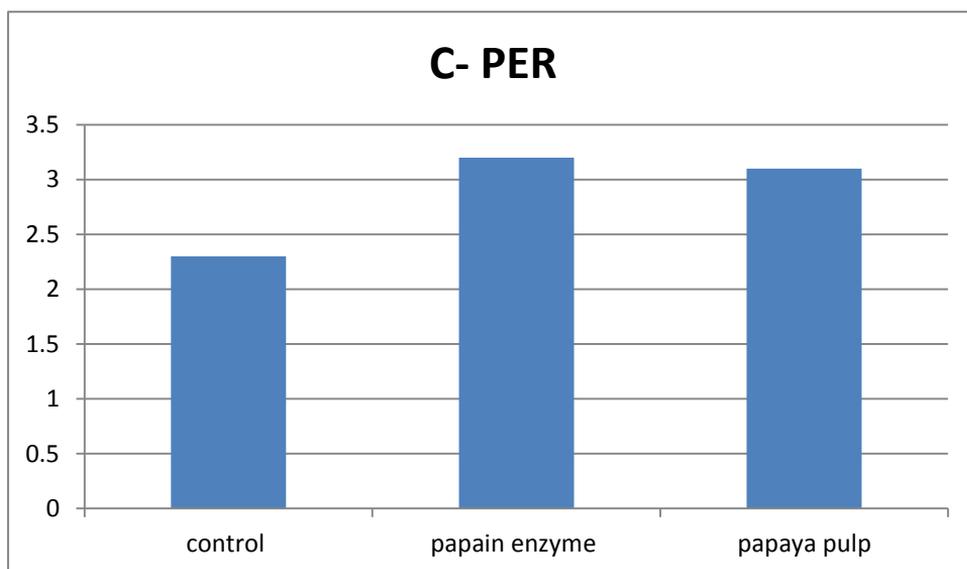


Fig. (3): Calculation of protein efficiency ratio (C-PER) of infant cereals flakes products

Table (2): Physicochemical characteristics of ripe papaya fruit pulp

Parameters	Values
Moisture (%)	87±0.08
Non-enzymatic browning (Optical density at 420 nm)	0.11±0.0
Viscosity SP	3.4 ±0.06
Acidity (as % anhydrous citric acid)	0.33±0.0
Total sugars (%)	8.69±0.06
Reducing sugars (%)	6.40±0.04
pH	4.87±0.01
Vitamin C (mg/100g)	46.55±0.4
Total carotenoids (mg/100g)	4.29±0.2
Color values	
L	37.51±0.02
a	9.15±0.04
b	23.47±0.02

*Means of triplicate ±SD.

Table (3): Sensory evaluation of infant cereals formulas

Products Characteristics	Control	With papaya pulp 5%	With papain enzyme 0.3%	LSD
Color(10)	7.7 ^b ± 0.18	9.0 ^a ± 0.19	7.3 ^c ± 0.15	0.4
Flavor (10)	7.8 ^b ± 0.14	9.0 ^a ± 0.15	7.5 ^b ± 0.17	0.3
Taste (10)	7.2 ^c ± 0.19	8.9 ^a ± 0.13	7.8 ^b ± 0.17	0.6
Oder (10)	8.0 ^b ± 0.16	8.9 ^a ± 0.73	8.1 ^a ± 0.18	0.1
Rehydration (10)	8.4 ^a ± 0.17	8.8 ^b ± 0.19	8.2 ^b ± 0.19	0.2
Overall acceptability (10)	8.1 ^b ± 0.17	9.2 ^a ± 0.18	8.9 ^b ± 0.18	0.6
Category	Good	Very Good	Good	

LSD significant = $p < 0.05$

Table (4): Physicochemical characteristics of control cereal flakes versus added papain enzyme and papaya pulp formula

Parameters	Control cereal flakes	Cereal flakes with Papain enzyme 0.3%	Cereal flakes with Papaya pulp 5%
Moisture (%)	3.00±0.01	3.02±0.01	3.08±0.01
Non-enzymatic browning (Optical density at 420 nm)	0.13±0.04	0.13±0.04	0.14±0.00
Acidity (as % anhydrous citric acid)	0.48±0.01	0.50±0.01	0.54±0.01
Total carbohydrate	71.12±0.6	71.22±0.6	68.72±0.5
Fat	5.76±0.2	5.74±0.2	6.28±0.2
Protein	15.00±0.3	15.13±0.3	14.63±0.3
Total sugars (%)	67.22±0.4	64.18±0.4	60.52±0.6
Reducing sugars (%)	58.26±0.3	58.06±0.4	56.78±0.8
Vitamin C (mg/100g)	12.48±0.3	12.48±0.4	41.61±0.4
Total carotenoids (mg/100g)	1.63±0.05	1.63±0.05	7.34±0.2
Color values			
L	73.30±0.04	70.22±0.03	54.73±0.02
a	6.55±0.02	6.80±0.01	8.11±0.04
b	18.41±0.01	21.14±0.02	30.30±0.02

*Means of triplicate ±SD.

Table (5): Vitamins and minerals composition of infant cereal flakes with papaya pulp on dry weight base per 100 g comparing with RDA*

Parameters	Control	With papain enzyme 0.3%	With Papaya Papaya pulp 5%	RDA for Infant
Vitamin contents				
Vitamin A I.U	440	440	1066	1300
% of RDA	34%	34%	82%	
Vitamin D ₃ I.U	250	250	250	200
% of RDA	125%	125%	125%	
Vitamin E I.U	3.2	3.2	3.5	4
% of RDA	80%	80%	88%	
Niacin (B3) mg.	2	2	3	3
% of RDA	67%	67%	100%	
Thiamin (B1) mg.	0.3	0.3	0.4	0.3
% of RDA	100%	100%	133%	
Riboflavin(B2) mg.	0.3	0.3	0.5	0.4
% of RDA	75%	75%	125%	
Vitamin B ₆ mg.	0.3	0.3	0.3	1.8
% of RDA	17%	17%	17%	
Vitamin B ₁₂ µg.	0.2	0.2	0.2	0.4
% of RDA	50%	50%	50%	
Vitamin C	12	12	41	50
% of RDA	24%	24%	82%	

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Minerals contents				
Calcium mg.	220	220	317	400
% of RDA	55%	55%	79%	
Iron mg.	10	10	10	11
% of RDA	91%	91%	91%	
Phosphorous mg.	211	211	322	460
% of RDA	49%	49%	70%	
Potassium mg.	280	280	318	700
% of RDA	40%	40%	45%	
Chloride mg.	350	350	470	570
% of RDA	61%	61%	83%	
Sodium mg.	81	81	97	370
% of RDA	22%	22%	26%	
Zinc mg.	3	3	3	4
% of RDA	75%	75%	75%	
Magnesium mg.	40	40	50	80
% of RDA	50%	50%	63%	
Iodine µg.	60	60	60	90
% of RDA	67%	67%	67%	
Copper µg.	300	300	300	340
% of RDA	88%	88%	88%	
Manganese µg.	60	60	100	80
% of RDA	75%	75%	125%	

*WHO 2011

Table (6): Microbiological results for cereal flakes with papaya pulp

Analyses Items	Specifications	Results		
		control	Cereal flakes with papain enzyme 0.3%	Cereal flakes with papaya pulp 5%
Total Plate Count	$\leq 1\ 000$ CFU/g	Conform	Conform	Conform
Total Yeast and Mold	≤ 24 CFU/g	Conform	Conform	Conform
E. Coli	Negative	Conform	Conform	Conform
Salmonella	Negative	Conform	Conform	Conform
Staphylococcus	Negative	Conform	Conform	Conform

إنتاج أغذية أطفال مدعمة ببروتين القمح المحلل جزئياً

محمد رمضان محمد مسعود - عبد العظيم سيد عبد العظيم عبد اللطيف - محمد رشاد جودة
يوسف

معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية- جيزة - مصر

الملخص العربي

اجريت الدراسة الحالية لتحسين خصائص امتصاص البروتين في أغذية الأطفال الرضع المصنعة اساسا من القمح باستخدام انزيم البابين مقارنة باضافة لب ثمار الباباظ كمصدر للانزيم . وقد اظهرت النتائج ان درجة التحلل للبروتين تزداد بزيادة فترة التحضين حتى 60 دقيقة بعدها درجة التحلل تصل الى اقصى درجة 2، 14، 16% للكنترول، انزيم البابين و لب الباباظ على الترتيب . وقد اظهرت النتائج ان اغذية الاطفال التي انتجت باضافة انزيم البابين وباضافة لب ثمار الباباظ كمصدر للانزيم كانت افضل من حيث المحتوي من المغذيات وكذا كفاءة البروتين و الجودة مقارنة بالكنترول. كما وجد ان العينات المضافة إليها لب الباباظ زياد محتواها من بعض المغذيات مثل البيتا كاروتين وبعض الاملاح المعدنية و الفيتامينات . لذا أوصت الدراسة باستخدامها في أغذية الأطفال الرضع ولبعض الحالات المرضية للبالغين وخاصة الذين يعانون من الحساسية.

الكلمات الدالة: القمح - الباباظ - بروتين قمح محلل - انزيم البابين - التجفيف بالاسطونات - أغذية الأطفال .