

CHEMICAL COMPOSITION AND SOME FUNCTIONAL PROPERTIES OF FLOUR AND ISOLATED PROTEIN FROM MUNG BEAN SEEDS (*Vigna radiate*) CULTIVATED IN IRAQ

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ABSTRACT

The objective of this research was to study the chemical composition and functional properties of flour and protein isolate from mung bean by isoelectric precipitation. Moisture, protein, fat, ash, crude fiber and carbohydrate were 5.88%, 24.52%, 1.24%, 3.33%, 5.22% and 59.81% for flour and 2.48%, 83.70%, 0.24%, 2.48%, 0.52% and 10.58% for isolated protein respectively. The results of functional properties revealed an increase in solubility rates by increasing the pH values of both flour and isolated protein, the mung bean flour showed higher solubility than protein isolate. The foam capacity of flour was higher than that of protein isolate, however foam stability for protein isolate reached 23.53%, in flour it decreased to 10% after 30 minutes. It was observed that emulsion formation was good for mung bean flour and isolated protein, while emulsion stability was better for protein isolate. Data of least gelation concentration showed that the protein isolate was able to form gel at 6% concentration, in flour it was noted at 8% and the increase of concentration improve gelation capacity for both. Finally mung bean isolate was treated with Flavourzyme to obtain hydrolysates at different degree of hydrolysis which were characterized for its ability to scavenge free radicals. It was observed that the antioxidant activity increased by increasing the time of hydrolysis, the activity was near to the effectiveness to ascorbic acid as a control after 4 hours. The most functional properties of protein isolate were good so it could be considered as potential functional food ingredients.

Keywords: Legume flour, characterization, protein hydrolysate.

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التركيب الكيميائي وبعض الخواص الوظيفية للطحين والبروتين المعزول من بذور الماش (*Vigna radiate*) المزروع في العراق

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المستخلص

هدف البحث دراسة التركيب الكيميائي وبعض الخواص الوظيفية للطحين والبروتين المعزول من بذور الماش بطريقة الترسيب بنقطة التعادل الكهربائي. بلغت نسب الرطوبة، البروتين، الدهن، الرماد، الالياف الخام والكربوهيدرات 5.88%، 24.52%، 1.24%، 3.33%، 5.22% و 59.81% في الطحين و 2.48%، 83.70%، 0.24%، 2.48%، 0.52% و 10.58% للبروتين المعزول على التوالي. اظهرت نتائج الخواص الوظيفية زيادة نسب الذوبانية بزيادة قيم الاس الهيدروجيني لكل من الطحين والبروتين المعزول. كانت سعة الرغوة للطحين اعلى من تلك التي للبروتين المعزول ولكن ثبات الرغوة للمعزول وصل 23.53% وانخفض في الطحين الى 10% بعد 30 دقيقة. لوحظ ان تكوين المستحلب كان جيداً لطحين الماش والبروتين المعزول وكانت ثباتية المستحلب افضل للبروتين المعزول مقارنة بالطحين. اشارت النتائج ان اقل تركيز للتهلم للبروتين المعزول كان عند تحضير محلول بتركيز 6% وفي طحين الماش عند تركيز 8% وان زيادة التركيز ادى الى تحسين قدرة تكوين الهلام لكليهما. عومل البروتين المعزول بانزيم Flavourzyme للحصول على متحللات في اوقات مختلفة ودرست قابليتها على كبح الجذور الحرة. لوحظ زيادة الفعالية بزيادة وقت التحلل وكانت الفعالية المضادة للاكسدة مقارنة لفعالية حامض الاسكوربيك كنموذج سيطرة بعد 4 ساعات من التحلل. معظم الخواص الوظيفية للبروتين المعزول كانت جيدة ولذلك يمكن اعتباره مكون غذائي ذو وظائف مهمة.

الكلمات المفتاحية: طحين البقوليات، توصيف، متحلل البروتين.

INTRODUCTION

In developing countries the average protein intake is less than that required, therefore plant proteins play significant role in human nutrition (17). Mung bean is one of the important legume seeds. *Vigna radiata* is summer pulse crop with short duration and high nutrition value. The beans are main source of amino acids as well as rich protein content, hence its easy to prepare protein isolates from this crop (27). Plant protein concentrates or isolates are commonly prepared by alkaline extraction then the extracted protein precipitated either by decreasing the pH to isoelectric point or by heating (21). High percentage of protein contents in protein isolates obtained from the legumes through precipitation will make potential protein sources for food industry applications and this potential benefit will depend on their functional properties (8). Functional properties are chemical and physical characteristics that influence the protein behavior in food system(27). Plant protein isolates are the most refined forms of proteins, they are often improve taste and appearance when compared with the original meal so they can be better used as nutritional and functional ingredients in many food products(2). Mung bean protein isolates were used in bread flour mixture as protein supplement. Isolated protein from mung bean show improvement in many desired functions such as foaming, emulsification and water absorption in processed food. The improvements in those function will make it more desirable as a food component (9). Enzymatic hydrolysis of proteins used to release bioactive peptides, and is widely applied to improve functional and nutritional properties (11). The aims of research were studying the chemical composition and functional properties of flour and isolated protein from mung bean seeds which may help to determine the potential application of them in food products, also to produce hydrolysates from protein isolate using Flavourzyme to evaluate their effect as antioxidant.

MATERIALS AND METHODS

The mung bean seeds (*Vigna radiata*) used in this research were obtained from Department

of field crops - College of Agriculture, University of Baghdad.

Preparation of mung bean flour

Mung bean seeds were cleaned manually to remove dust, branches and foreign materials, washed with tap water and dried on air at room temperature. The whole seeds were ground to powder (0.6 mm) form with grinder, the flour stored in a plastic container at (- 20° C) until used.

Preparation of protein isolate by isoelectric precipitation

Protein isolate was prepared from seeds flour following the method described by Nasriyanti et al(20). The flour was dispersed in distilled water in a 1:15 (w/v) ratio, the pH of the suspension was adjusted to 9 using 1N NaOH. The materials were mixed for 20 minutes at 25° C using an incubator shaker. Insoluble components were removed by centrifuged at 1000 xg for 20 minutes. The protein in the extract was precipitated at pH 4 using 1N HCl. The precipitate was separated by centrifugation at 1000 xg for 20 minutes and washed once with distilled water and readjusted to pH 7 with 1N NaOH and dried in an oven at 40°C for the next day.

Determination of proximate composition

Standard methods of analysis according to AOAC (4) were used for determination percentage of moisture, fat content, crude fiber, ash and protein content determined using micro-Kjeldahl method (%N X 6.25). Carbohydrate (%) was determined by difference .

Functional properties of mung bean flour and protein isolate

Protein solubility : The solubility percentage was determined according to the suggested method by Hindi (13). 1 gm of samples powder was mixed with 100 ml of distilled water, the pH of the solution was adjusted to 5 , 6 , 7, 8 with 1N HCl or 1N NaOH. The mixture stirred by magnetic stirrer for 45 min. at 30°C, after that 2ml of the filtrate was taken to estimate its content of total soluble nitrogen (Ps) by micro-Kjeldahl method, and its content of total protein (Pt) The percentage of solubility was calculated as follows:

$$\text{Solubility \%} = (\text{ps} / \text{pt}) \times 100$$

Emulsion properties : The protein ability to make emulsion and its stability was

determined using the suggested method by Hindi (13). 1 gm of the sample mixed with 50 ml of distilled water and 10 ml of corn oil homogenized for 2 min and held in a graduated cylinder at 25°C, the stability of emulsion was observed after 3, 6, 9, 12, 24 hours.

Foaming properties

The ability of protein to make foam and its stability was estimated according to the method of Jasim (14) with some modification by mixing 1gm of the sample with 100ml of distilled water using mixer at a speed of 10000 rpm for 3min, then the foam volume before and after 20 sec. mixing was recorded, also foam volume after 10,30,60 and 90 minute was read.

Foam capacity % =

$$\frac{\text{foam's volume at zero time after mixing} - \text{foam's volume before mixing}}{\text{foam's volume before mixing}} \times 100$$

Foam stability % =

$$\frac{\text{foam's volume after time (t)}}{\text{foam's volume at zero time after mixing}} \times 100$$

(El-tayeb et al, 2011)

Least Gelation concentration

The LGC was determined by appropriate sample suspension of 4, 8, 10, 12, 14, 16, 18, 20% (w/v) prepared in 5ml distilled water. The test tubes containing these suspension were heated for 3 hours in boiling water followed by cooling under running water, and further cooled at 4°C. LGC is the minimum concentration at which the gel did not slide along the test tube walls in inverted position (22).

Preparation of protein hydrolysate

Protein hydrolysate was prepared according to Parado et al (23) method. Isolated protein was dispersed in distilled water in the ratio of 5:100 (w/v) in beaker. The pH of suspension was adjusted to 7 using IN NaOH the beaker was covered and placed in shaker water bath

fixed on 55°C previously for 10 min. The hydrolysis began by adding Flavurzyme (obtained from sigma –Aldrich) at the rate of 1.5 ml enzyme/ 100 ml of suspension (enzyme activity 300 U/ ml) and incubated for 1, 2, 4 hours. Hydrolysis was stopped by transferring the beaker to another water bath at 90°C for 10 min. to inhibit enzyme activity in the hydrolysate, centrifuged at 4000xg for 10 min. then the filtrate was freeze – dried for further use.

DPPH Radical scavenging activity

Scavenging activity of the samples were determined according to the method of Bersuder et al (7) with slight modification using 2,2-diphenyl -1-picrylhydrazyl (DPPH). The solutions were mixed vigorously with methanol 99.5% containing DPPH, and kept in dark at room temperature for 30 min. then the absorbance was measured at 517 nm. The antiradical activity was calculated as follows:

$$\text{Radical scavenging activity \%} = \frac{\text{DPPH Blank} - (\text{DPPH sample} + \text{control sample})}{\text{DPPH Blank}} \times 100$$

Where DPPH Blank= absorbance of 4 ml distilled water + 1 ml of 0.1Mm methanolic DPPH solution.

DPPH sample= absorbance of 4 ml aqueous sample + 1 ml of 0.1Mm methanolic DPPH solution.

Control sample= absorbance of 4ml aqueous sample + 1ml methanol.

The control prepared by mixing 4 ml distilled water + 1ml methanol.

RESULTS AND DISCUSSION

Chemical analysis : The proximate chemical analysis is very important in determining the quality and nutritional value of food. flour of seeds cultivated locally in Iraq and protein isolate were analyzed for the chemical composition, the results of these analysis are given in table 1.

Table 1. proximate composition of mung bean seeds and isolated protein ⁽¹⁾

	Moistuer %	Fat %	Crude Fiber %	Ash %	Carbohydrate ⁽²⁾ %	Protein %
Flour	5.88	1.24	5.22	3.33	59.81	24.52
Isolated protein	2.48	0.24	0.52	2.48	10.58	83.70

(1) Average of duplicate .

(2) Obtained by the difference

It was observed that the moisture content of flour was 5.88 %. Results from other reseach pointed out that moisture range was between 8.25 – 12.07g /100g, which is much higher than our value (24). The crude fat content was

also low with a value of 1.24%. No attempt was made to remove the crude fat because the fat content value is relatively low and additional step to extract oil would be time consuming and increase production costs,

while other researchers recorded high fat content (9). The crude fiber content was 5.22 %, the reason for such a high fiber is the included hull in the analyzed sample. Augustin and Klein (5) mentioned that fiber content for green mung bean between 1.6 to 3.2 % , and the high percent of fiber content make the mung bean as a good digestive source. The flour content of ash in this study was 3.33% which was similar to 3.34% recorded by Agugo and Onimawa (1) . However Paul et al (24) reported higher ash content 3.85%. Ash content in the mung bean indicates that the seeds provides essential minerals. The carbohydrate percentage was 59.81% which is near to the value 60.35% reported by Paul et al (24), but lower than the value 62.3% reported by Mubarak (19). Total protein content of flour was 24.52% the range being between 27-32.86% (9). The observed differences in chemical composition of seeds may be due to differences in environment conditions and species, also different analysis methods. The percentage of fat decreased to 0.24% in the isolated protein, this will help in increase keeping quality. This result indicating that most of fat was removed during protein isolation process, also the levels of ash and crude fiber were reduced. Generally carbohydrate percentage in flour was higher than isolated protein (10.58%), on contrast the latter has higher protein content (83.70%). Flour was used as a starting material for the preparation of isolated protein. Result indicated that isoelectric precipitation method can improve the protein content. It was suggested that isolated protein from mung bean flour could be considered as an additional source of plant protein in food product (17). The protein isolate is relatively pure protein

since most of the soluble constituent are removed especially oligosaccharides, thus the dark colours of proteins extracted under alkaline conditions are probably due to oxidative products of alkaline stable phenolic components .

Functional properties

Solubility property: The solubility considered as the most important and critical property of functional properties since it affects other properties, and a good indicator for evaluating the potential application of proteins (16). Figure 1 appears the solubility capability of isolated protein from mung bean, the percentage of solubility were 27%, 42.27% 52.45% and 60.23% at pH values 5, 6, 7, 8 respectively, while the percentages of mung bean flour solubility were 42.2%, 61.35% , 77.46% and 88.53% at the same pH values, which shows that the solubility of mung bean flour was higher than isolated protein. This may be due to affinity of higher polar amino acids residues of proteins to water molecules. Proteins and carbohydrates are the major chemical composition of flours that contain hydrophilic parts, such as polar or charged side chain (10). There was a decrease in solubility both of flour and isolated protein at the pH 5 as it is the lower solubility because it is the pH near to iso electric point in mung bean protein. There was an increase in the solubility with the increase of pH value and this is due to the electrical forces between protein groups charged negative which help to keep these groups distant and increase of solvent protein interaction (14). Conditions that shift equilibrium in favour of protein -solvent interactions increase the solubility and conditions that favour protein - protein interaction decrease the solubility (12).

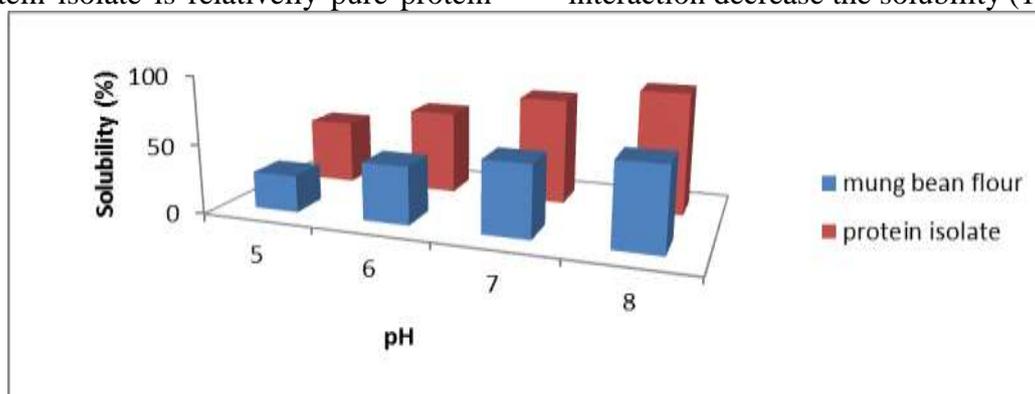


Figure 1. Effect of pH values on the protein solubility (%) of mung bean flour and protein isolate

This result was in agreement with Martinez - Flores et al (18) who stated that the soluble of flax seed protein isolate and concentrate was high at alkaline pH and reached more than 80% at pH 10. It was less solubility at pH 5 which is near to iso electric point of flax seeds protein (4.8) and reached 18%. Khalid and Elharadallon (15) also reported that the solubility of the isolated protein from cowpea and lupin was good in both acid and alkaline pH regions, for food formulation it considered as the most important characteristics so seed protein could find good applications in acidic beverages and soft drinks in order to improve and increase its nutritional quality and protein content.

Foam properties

Foam plays an important role in food industry and saves a desired and unique structure for a group of foods such as ice cream , cake , bread and soft drinks. The stability of food's foam is necessary to be acceptable by consumer, the quality of product could be also affected by appearance. Figure (2) shows foam properties of isolated protein and mung bean flour in pH 7 at room temperature, it seems that the

volume of the composed foam for isolated protein was 17 ml while it increased up to 50ml for the flour this could be referred to fact that the protein in flour are surface active. The globular nature of the protein may cause the poor foaming capacity of isolated protein which reduced the ability to foam interfacial membrane around the air bubbles (3). The results shows with regard to the stability of foam that it decreased gradually until 30min in mung bean flour. The foam stability of isolated protein was better, it reached 23.53% while foam stability of flour reduced to 10% after 30minutes. It seems that the relationship between foam capacity and foam stability was an inverse. High foaming ability of flours could form large air bubbles surrounded by thinner layer and less flexible protein film, so the air bubbles might be easier to collapse and lowered the foaming stability (10). This result suggested that the protein structure in isolated protein was more flexible in aqueous solution and interacted strongly at the air – water interface to form more stable foams compared to flour(17).

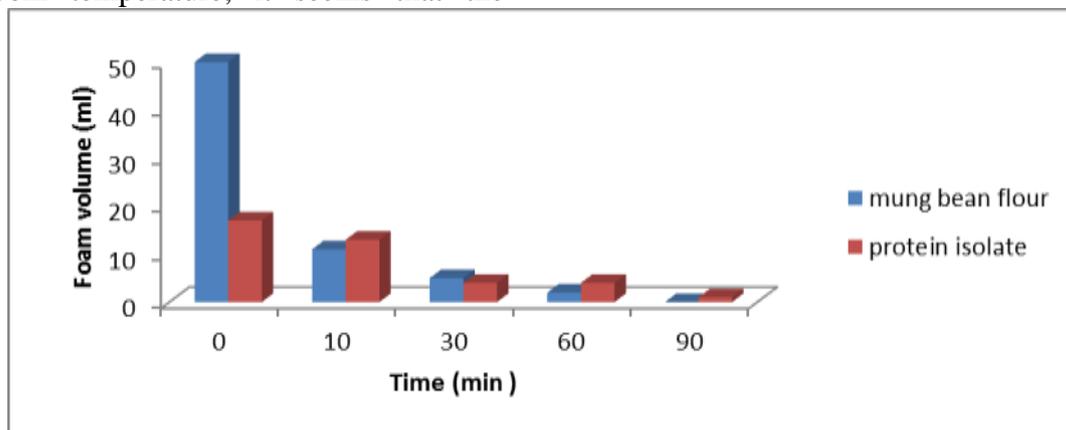


Figure 2. Foam stability of mung bean flour and protein isolated

Emulsifying properties: The emulsion is distribution of oil drops in the continuous liquid phase, emulsifying is generally a kind of oil in the water although some of the foods such as butter and margarine are kinds of water in fat. The activity of emulsion soluble protein depend on the equilibrium between hydrophobic and hydrophilic groups at the interface of oil –water, the hydrophobic groups in the protein will tend to fat phase, and the hydrophilic groups to the water phase, so the surface tension will reduce at the interface. Formation and stability of emulsion of mung bean flour and protein isolate at pH 7 in room

temperature are presented in table (2), the results show reducing of emulsion layer by time passing in contrast increasing of water layer. After 24 hours the isolated protein emulsion layer was 29 ml and water layer 31ml while the emulsion layer of flour was 25 ml and the water layer was 35ml, so the stability of protein isolate emulsion is better than the flour, the reason of that may be due to the differences in their protein content and hydrophobicity of surface (17). Emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domain (16).

Table 2. Emulsion stability of mung bean flour and protein isolae

Time / hour	Protein isolate		Flour	
	Water layer (ml)	Emulsion layer (ml)	Water layer(ml)	Emulsion layer(ml)
0	0	60	0	60
3	0	60	0	60
6	1	59	2	58
9	13	47	18	42
12	29	31	34	26
24	31	29	35	25

According to these results the emulsion formation property was good for mung bean flour and protein isolate while the emulsion stability was better for isolated protein. Stability of emulsion expressed as ability of emulsion to resist the changes in its properties for long possible period. Pawar et al (25) explained that the protein isolate of sunflower has good emulsifying properties in composing the emulsion and its stability.

Gelation

The gels are three-dimensional matrices that retain water, lipids, sugar, flavours and other ingredients, ability to forming gels is very useful in new product development (26). The

results of least gelation concentration in table (3) shows that both mung bean flour and protein isolate were not able to form gel at lower concentration 4%, but it was noted at 8% for flour and 6% for protein isolate. Eltayeb et al (10) pointed out that gelation formation of Bambara flour and protein isolate was observed at 8 and 18% respectively. Generally increase in flour and protein isolate concentration improved gelation capacity, this improvement because of decrease in thermodynamic affinity of proteins for the aqueous solution which increase the interaction between proteins (10).

Table 3. Least gelation concentration of mung bean flour and protein isolate

(-) no gelation (+) gelation

Percentage protein concentrate	Mung bean flour	Protein isolate
4	-	-
6	-	+
8	+	+
10	+	+
12	+	+
14	+	+
16	+	+
18	+	+
20	+	+

Gelation is an aggregation of denatured molecules. The variation in gelling properties between flour and protein isolate may be due to variation in the relative ratio at different constituents such as protein, lipids and carbohydrates, and the interaction between such components may affect functional properties (6 , 26). The ability of protein to form gels is useful in food application and in new product development, thereby providing an added dimension to protein functionally (10).

Anti – oxidant activity

An experiment was done to know the ability of protein isolate and protein hydrosolysates from mung bean produced by the treatment with Flavourzyme to show antioxidant capacity in scavenging free radicals at different hydrosolysis degree. Figure 3 appears that isolated protein had antioxidant activity 40.36% at zero time.

After enzymatic hydrolysis the antioxidant activity increased by increasing the time of hydrolysis it was 58.29 %,74.38 % after 1,2 hours respectively and reached the maximum after 4 hours, it was 77.62%. The reason for this may be attributed to the increase of short peptides chains with bioactive functional due to the enzyme hydrolysis. While the antioxidant activity of ascorbic acid as a comparative antioxidant in concentration 100mg / ml was 80.47%. The results (of this experiment) pointed out that the enzymatic hydrolysate after 4 hours hydrolysis has antioxidant activity near to ascorbic acid as a control sample. It can be concluded that it can be used in the food as natural antioxidant factors alternative of artificial antioxidant which may cause side effects, in addition these hydrolysate from protein origin add nutritional value of the products.

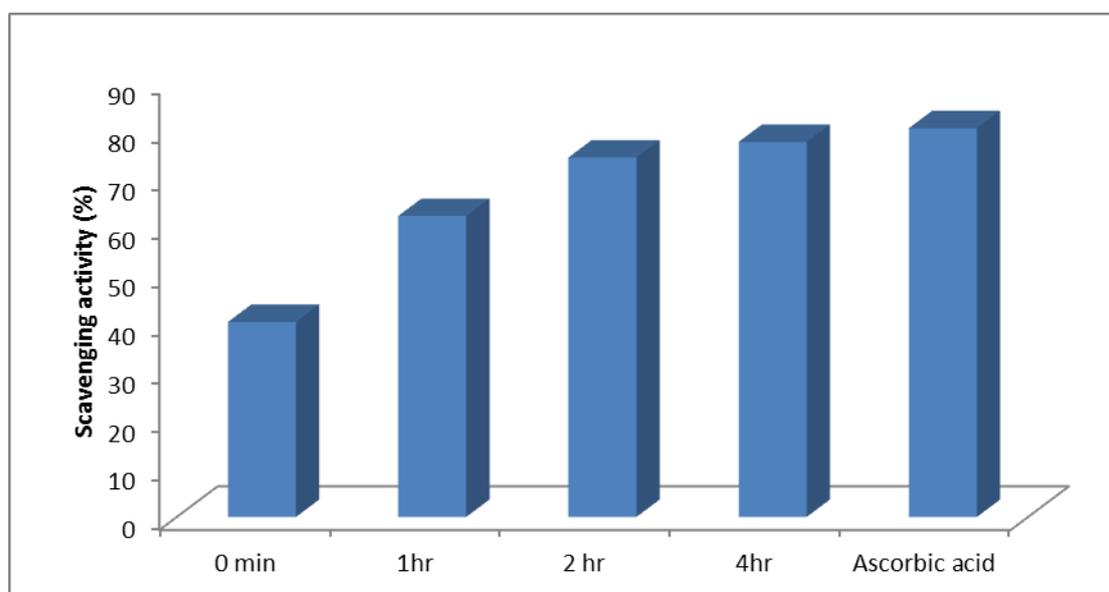


Figure 3. DPPH scavenging activity(%) of mung bean protein isolate hydrolyzed with Flavourzyme

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