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Impacts of Enzymatic Modifications of Legume's [i.e., Chickpea (*Cicer arietinum*) and Lentil (*Lens culinaris*)] Protein Extracts on Pasta Functional Characteristics"

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ABSTRACT

This study investigated the effect of enzymatic hydrolysis of chickpea and lentil protein extracts on pasta functional characteristics. Wheat flour was substituted with 0, 5, 10, 15,and 20% of hydrolyzed protein extract and non-hydrolyzed extracts from lentils and chickpea flours. Study treatment includes sequential enzymatic followed by acid and then alkaline treatments. Enzymatic hydrolysis of legume protein extracts had significant impacts on legume functional characteristics increase in legume protein hydrolysis resulted in a linear increase in water absorption regardless of enzymatic hydrolysis and/or chemical extraction method. Results showed a decrease in water absorption with protein hydrolysis while enzymatic hydrolysis before protein extraction treatment resulted in the greatest water absorption among treatments. Additionally, chickpea and lentil treatments impacted the treated farinograph resulting in faster arrival time with the enzymatic modification. For instance, arrival time decreased from 138 to 318% for lentils and from 123 to 183% for chickpea indicating a faster dough developing time that would contribute to saving in pasta processing time. Cooking loss of pasta during cooking decreased from 2.5% in the control treatment to 1.2% (i.e., 208% reduction) in the acid, alkaline treated chickpea and to 0.4% (i.e., 625% reduction) in treated lentil samples. The use of Papain (EC 3.4.22.2) enzymatic treatment was the most effective in decreasing the cooking loss of cooked pasta. Cooked pasta hardness also decreased from 2010.6 N in the control sample to 97.7, 39.9 and111.7 N for acidic, sequential acid then for the alkaline treatment of chickpea, respectively. Similar results were reported for lentils with hardness ranging from 48.1, 47.1 to 50.0 N. Therefore, the use of enzymatic modifications would contribute to improved pasta quality characteristics

Keywords: Pasta, functional characteristics, chickpea flour, lentil flour.

INTRODUCTION

Pasta is usually consumed as a convenience, palatable and nutritional food product having more than 75% carbohydrates and 12% proteins (Bashir *et al*., 2012). Pasta and other cereal products are typically manufactured from durum wheat which makes them deficient in lysine and threonine (Messia *et al*., 2021). Therefore, supplementation of pasta with these amino acids–rich legume flours is of great interest to increase its nutritional quality. Pulses generally have low levels of

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sulfur-containing amino acids and tryptophan but are rich in lysine. Several studies were published regarding the supplementation of pasta flour with various bowls of cereals and legumes (Laleg *et al*., 2021). Zhao *et al*., (2005) in the same manner reported the negative impacts of untreated green and yellow peas, lentils, and chickpea flours on the functionality of spaghetti with the increase in degree of substitution.

Cooked pasta texture is the key factor of pasta quality (D'Egidio *et al.,* 1990). Hydration kinetics (i.e., affecting cooked pasta firmness), and solids leaches (i.e., affects cooked pasta stickiness) of dough during pasta processing play critical roles in constructing the final pasta quality (Bresciani, *et al*., 2022). Therefore, the ability of pasta ingredients to entrap water and limit the solids leach could determine the cooked past's quality (Bianchi *et al*., 2021). More specifically, the ability of such ingredients to form and stabilize a continuous protein-starch-lipid network can provide the necessary cohesion of pasta (Schmid, *et al*., 2023).

The hydrolysis of pasta flour proteins during processing is essential to enhance the protein's binding ability for water including pasta quality (Petitot *et al*., 2009). The concentration of the dispersed phase is believed to increase; providing suitable conditions for starch–protein complex formation (Wang *et al*., 2020). Furthermore, protein hydrolysis/extraction using mild heat treatment before processing is expected to reduce protein's intermolecular interactions and also increase the amount of available water for starch; thus increasing the leaching of more soluble amylose and increased stickiness during cooking; a detrimental property of cereal products (Debbouz and Doetkott 1996).

Pulses including chickpeas and lentils are gaining interest as healthy food choices due to their high protein, fiber, starch, vitamin, and mineral contents (Gurusamy *et al*., 2022). The use of legumes in cereal products, however, appears to be restricted because of factors including low protein and starch digestibility and the content of significant amounts of anti-nutritional factors that might be distributed ubiquitously within plant foods including vegetables, cereals, legumes and fruits, especially when used as wholegrain (Joye I. 2019). Chickpeas, for instance, contain trypsin inhibitors, phytic acid and tannins that can reduce the availability of amino acids and affect growth (Samtiya *et al*., 2020). Similarly, the presence of phenolic compounds and their oxidized products in lentils can also lead to the formation of complexes with essential amino acids, enzymes, and other proteins, thus lowering their protein digestibility and nutritional values (Mustafa *et al*., 2022). Protein hydrolysis/extraction, therefore, is essential to either eliminate or disrupt its undesirable components to improve its functionality and to be utilized as an ingredient in cereal products.

Although, the use of heat treatment to improve legume protein's functionality is reported; limited information is currently available on using low-temperature protein hydrolysis/extraction on flour functional properties. The author's hypothesis is that disruption of protein through extraction and subsequent hydrolysis using enzymes would change chickpea and lentil flour's functional characteristics. Therefore, the objective of this work is to investigate the effects of enzymatic hydrolysis of disrupted legume proteins through acidic and alkaline extraction protocol on the functional and quality characteristics of pasta as a model system, made using various fractions of treated chickpea and lentil flours.

Materials and Methods

Materials and experimental design

Wheat flour, chickpea (*Cicer arietinum*), and Lentil (*Lens culinaris*) used in this study were purchased from a local market in Amman, Jordan. Treatments used for pasta manufacturing include various fractions of treated and non-treated chickpea and lentil flours. Acidic (pH=2) and alkaline (pH=10) were adjusted using HCl and NaOH, respectively (pH meter No. 40675/0001: HANNA Instrument, United Kingdom *Aspergillus saitoi* (EC 3.4.23.18 - Aspergillopepsin I, P2143-25G, 1001396035, Japan) and Papain proteinase (EC 3.4.22.2- papain, P3250-25G, 1001465678, Japan) were used to hydrolyze chickpea and lentil flours. Sequential acid, alkaline protein extraction and enzymatic hydrolysis treatments

were also performed to hydrolyze chickpea and lentil proteins that are used for pasta manufacturing. Treatments were made by incorporating and thoroughly mixing fractions of treated and/or non-treated chickpea and lentil flours in ratios of 5, 10, 15, and 20% in wheat flour. A control sample of no replacements was included in the study. Replacements of greater than 20% were not included in the study to preserve pasta texture since greater replacement was found detrimental to pasta characteristics.

Preparation of protein extracts using an acidic-basic technique from chickpea or lentil

Proteins were extracted using acid and/or alkaline conditions from chickpea or lentil flour and were performed according to the method described by Saleh *et al*., 2015. In summary, suspended chickpea and lentil flours were and held at 40°C for 30 minutes using a water bath (Model WB 14, DIN176-1K1, Memmert GmbH+Co, KG, Germany). The pH of the solution was then adjusted using 0.2 M HCl to a pH of 2.0. Treatments were held at 40°C with continuous mixing for three hours. Acid-hydrolyzed flour was then terminated, and the pH was then adjusted to a pH of 10.0. using 0.2 M NaOH. Treatments were then terminated and cooled to room temperature (~23.2°C) and pH was adjusted to 6.0 - 6.2 using 0.1M HCl. Samples were then dried at 40°C using a drying oven (PRECISION, Winchester, VA, USA) to 12% wet bases moisture content and used for enzymatic treatments.

Enzymatic hydrolysis of protein extracts from chickpea and lentil flour

Chickpea and lentil flours were suspended in acid alkaline, or acid-alkaline treatment by adding 250 ml of distilled water per 50 g flour. The temperature and pH of the solution were adjusted to the optimum activity for each enzyme as described by the manufacturer (i.e., pH 5.4, 35°C for 2 hours for *Aspergillus saitoi* and pH 4.4, 42°C for 3.2 hours for Papaya proteinase enzymes) Enzymatic treatment durations varied according to the enzymatic reactions were terminated by adjusting the pH of the solution using NaOH and HCl solutions depending on the operating conditions of each enzyme. Treatment's pH was finally adjusted to 7.0 before drying to 12% wet bases moisture content using a drying oven (PRECISION, Winchester, VA, USA).

Farinograph measurements

Farinograph measurements were performed according to AACC method 54-21.02 (2000) using a farinograph (Model No. 8 10 101 (31, 5 and 63 rpm), Brabender, OHG, Duisburg, Germany). In summary, treatments of 300 g samples (i.e., on a 14 percent moisture basis) were weighed and placed into the corresponding farinograph mixing bowl. Water is then added to the flour and mixed to form dough and the amount of water added (i.e., water absorption) resulted in a Brabender Unit (BU) of 500 was recorded as the amount of water absorption. The curve is centered on the 500-Brabender unit (BU) line ± 20 BU by adding the appropriate amount of water and is run until the curve leaves the 500-BU line (Shewry *et al*., 2000).

Farinograph parameters include water absorption (i.e., the amount of water required to center the farinograph curve on the 500-Brabender unit (BU) line), peak time (i.e., the time in min. required to reach maximum consistency, arrival time (i.e., the time in min. when the top of the curve touches the 500-BU line), departure time (i.e., the time in min. when the top of the curve leaves the 500-BU line), stability (i.e., the difference in time between arrival time and departure time and mixing tolerance index (i.e., the difference in BU value at the top of the curve at peak time and the value at the top of the curve 5 min. after the peak) were measured.

Cooked pasta quality

Pasta making, and cooked pasta quality

Flour of protein hydrolyzed chickpea and lentils of various ratios were mixed with 30% (w/w) water containing 2 % salt for pasta dough making. A pasta-making machine fitted with an adjustable sheet thickness cutter was used to manufacture pasta. Raw pasta was cooked in excess boiling water for 6 minutes according to AACC method number 66-50.01, 2000. Cooked pasta

was then immediately drained, cooled ,and held at room temperature (23.2°C) for quality measurements. All analyses on cooked pasta were made during the one hour of pasta cooked.

Water uptake of cooked pasta

Water uptake of pasta was measured before and after cooking according to the following equation:

Water uptake (% db) =

$$\left(\frac{water\ content\left(cooked\ pasta\right)}{water\ content\left(dry\ pasta\right)}-1\right)x\,100$$

Cooked loss of pasta during cooking

A two stage drying procedure was used to calculate the cooking loss of pasta. Cooking loss was calculated using the following equation:

Cooking loss (% db) =

$$\left(\frac{dry\ matter\left(cooked\ pasta\right)}{dry\ matter\left(dry\ pasta\right)}-1\right)x\,100$$

Cooked pasta textural properties

Texture properties of cooked pasta were evaluated using a texture analyzer (Mecmesin Ltd, West Sussex, RH1306Z, UK) according to the method described by Saleh *et al*., (2017). A single compression test measurement of cooked pasta was performed using a 35 mm cylindrical probe compressing a single dough ring strand at a constant deformation rate of (1 mm/s) to 80% of the initial strand thickness. Hardness (i.e., the maximal peak force attained during the first compression) and stickiness (i.e., the negative area under the first compression curve) were recorded. Fiver measurements were performed for each treatment and averages were reported.

Sensory attributes for cooked pasta

Sensory attributes of cooked pasta were conducted in the sensory evaluation laboratory, Department of Nutrition and Food Technology, Faculty of Agriculture, the University of Jordan. A total of 75 consumers were randomly selected and performed the sensory testing using a 9-point hedonic scale according to Meilgaard *et al*., (2007). Reference pasta sample was included in as the no treatment sample. Consumers were also asked to intensify the overall product firmness, stickiness, flavor and color of each sample.

Statistical analysis

All measurements were performed in duplicate and mean values were reported. Analysis of variance (ANOVA) using JMP (release 10, SAS institute, Cary, NC) was performed to determine any significant differences among the treatment parameters associated with the pasta functional characteristics. Least significant difference (LSD) at a 5% level of probability was determined to separate differences in the properties among treatments.

Results and Discussion

Tables 1 and 2 present the farinograph water absorbance (%), arrival time (min.), departure time (min.), stability time (min.), peak time (min.) and mixing tolerance index (%) of chickpea and lentil disrupted and fractional treatments, respectively.

Table 1: Farinograph parameters of wheat flour substituted with different ratios of enzymatically hydrolyzed protein extracts from chickpea flour.

Enzyme Treatment	Acidic protein extract				Acidic and Alkaline protein extract				Alkaline protein extract			
	Fraction Replacement (%)											
	5	10	15	20	5	10	15	20	5	10	15	20
	Water Absorption (%)											

Papain (EC 3.4.22.2)	56.4 ^{c}	57.0 ^{c}	60.2 ^{c}	61.9 ^{b}	66.0 ^{a}	66.0 ^{b}	66.4 ^{b}	67.0 ^{b}	63.0 ^{a}	63.7 ^{a}	66.2 ^{a}	67.1 ^{a}
Aspergillus saitoi (EC 3.4.23.18)	62.8 ^{a}	64.9 ^{a}	64.5 ^{a}	64.5 ^{a}	66.0 ^{a}	67.0 ^{a}	68.0 ^{a}	68.0 ^{a}	62.0 ^{b}	63.7 ^{a}	64.0 ^{b}	65.1 ^{b}
Control	59.8 ^{b}	60.8 ^{b}	61.0 ^{b}	61.9 ^{b}	59.8 ^{b}	60.8 ^{c}	61.0 ^{c}	61.9 ^{c}	59.8 ^{c}	60.8 ^{b}	61.0 ^{c}	61.9 ^{c}
Arrival time (min)												
Papain (EC 3.4.22.2)	1.2 ^{b}	0.7 ^{c}	1.2 ^{b}	1.3 ^{b}	1.2 ^{b}	1.3 ^{b}	1.4 ^{b}	1.4 ^{b}	1.0 ^{b}	1.2 ^{b}	1.2 ^{b}	1.4 ^{b}
Aspergillus saitoi (EC 3.4.23.18)	1.2 ^{b}	1.3 ^{b}	1.3 ^{b}	1.2 ^{b}	1.2 ^{b}	1.1 ^{c}	1.2 ^{c}	1.1 ^{c}	1.0 ^{b}	1.2 ^{b}	1.2 ^{b}	1.1 ^{c}
Control	1.8 ^{a}	1.8 ^{a}	2.0 ^{a}	3.5 ^{a}	1.8 ^{a}	1.8 ^{a}	2.0 ^{a}	3.5 ^{a}	1.8 ^{a}	1.8 ^{a}	2.0 ^{a}	3.5 ^{a}
Departure time (min)												
Papain (EC 3.4.22.2)	7.2 ^{c}	10.2 ^{b}	9.7 ^{b}	8.2 ^{b}	9.3 ^{a}	8.3 ^{b}	8.2 ^{b}	8.1 ^{b}	9.2 ^{b}	8.1 ^{b}	7.4 ^{c}	7.2 ^{c}
Aspergillus saitoi (EC 3.4.23.18)	12.6 ^{a}	13.6 ^{a}	19.4 ^{a}	11.4 ^{a}	7.7 ^{c}	12.2 ^{a}	14.1 ^{a}	18.2 ^{a}	13.2 ^{a}	11.4 ^{a}	15.0 ^{a}	13.6 ^{a}
Control	8.6 ^{b}	8.2 ^{c}	8.7 ^{c}	8.4 ^{b}	8.6 ^{b}	8.2 ^{b}	8.7 ^{b}	8.4 ^{b}	8.6 ^{c}	8.2 ^{b}	8.7 ^{b}	8.4 ^{b}
Stability time (min)												
Papain (EC 3.4.22.2)	5.9 ^{c}	9.2 ^{b}	8.3 ^{b}	7.0 ^{b}	7.4 ^{a}	6.3 ^{b}	6.5 ^{c}	6.3 ^{c}	7.7 ^{b}	6.7 ^{b}	6.2 ^{c}	5.4 ^{c}
Aspergillus saitoi (EC 3.4.23.18)	11.2 ^{a}	12.2 ^{a}	17.9 ^{a}	19.4 ^{a}	6.4 ^{b}	10.9 ^{a}	12.5 ^{a}	17.1 ^{a}	12.2 ^{a}	10.0 ^{a}	13.5 ^{a}	12.1 ^{a}
Control	7.2 ^{b}	6.6 ^{c}	7.2 ^{c}	7.2 ^{b}	7.2 ^{a}	6.6 ^{b}	7.2 ^{b}	7.2 ^{b}	7.2 ^{b}	6.6 ^{b}	7.2 ^{b}	7.2 ^{b}

[1] Means of Farinograph parameters of wheat flour replaced with the same fraction of treated chickpea flours having the same chemical treatment and various enzymatic treatments with different letters are significantly ($P<0.05$) different according to least squares differences (LSD).

Various chemical and enzymatic treatments of chickpea and lentil flours resulted in significant ($P<0.05$), however, inconsistent results of farinograph parameters.

Table 2: Farinograph parameters of wheat flour substituted with different ratios of enzymatically hydrolyzed protein extracts from lentil flour.

Enzyme Treatment	Acidic protein extract				Acidic and Alkaline protein extract				Alkaline protein extract			
	Fraction replacement (%)											
	5	10	15	20	5	10	15	20	5	10	15	20
	Water Absorption (%)											
Papain (EC 3.4.22.2)	59.0 ^{c}	61.8 ^{c}	65.0 ^{b}	65.2 ^{b}	67.0 ^{a}	67.4 ^{a}	67.6 ^{b}	70.0 ^{a}	63.2 ^{a}	64.4 ^{a}	66.2 ^{a}	67.5 ^{a}
Aspergillus saitoi (EC 3.4.23.18)	67.3 ^{a}	64.8 ^{a}	66.8 ^{a}	68.1 ^{a}	67.0 ^{a}	67.0 ^{a}	68.0 ^{a}	69.0 ^{b}	62.3 ^{b}	63.2 ^{b}	64.5 ^{b}	66.0 ^{b}
Control	63.1 ^{b}	63.3 ^{b}	64.5 ^{c}	62.5 ^{c}	63.1 ^{b}	63.3 ^{b}	64.5 ^{c}	62.5 ^{c}	63.1 ^{a}	63.3 ^{b}	64.5 ^{b}	62.5 ^{c}

	Arrival time (min)											
Papain (EC 3.4.22.2)	1.2 ^{b}	1.1 ^{b}	1.3 ^{b}	1.2 ^{b}	1.2 ^{b}	1.2 ^{b}	1.3 ^{b}	1.2 ^{c}	1.3 ^{b}	1.2 ^{b}	1.3 ^{b}	1.5 ^{b}
Aspergillus saitoi (EC 3.4.23.18)	1.1 ^{b}	1.2 ^{b}	1.3 ^{b}	1.3 ^{b}	1.0 ^{c}	1.3 ^{a}	1.4 ^{b}	1.3 ^{b}	1.1 ^{b}	1.2 ^{b}	1.2 ^{b}	1.2 ^{c}
Control	1.7 ^{a}	1.6 ^{a}	2.0 ^{a}	2.2 ^{a}	1.7 ^{a}	1.6 ^{a}	2.0 ^{a}	2.2 ^{a}	1.7 ^{a}	1.6 ^{a}	2.0 ^{a}	2.2 ^{a}
	Departure time (min)											
Papain (EC 3.4.22.2)	8.2 ^{d}	9.3 ^{b}	8.2 ^{b}	9.2 ^{b}	8.9 ^{b}	8.4 ^{b}	8.3 ^{b}	6.3 ^{b}	8.4 ^{c}	8.1 ^{b}	7.3 ^{a}	7.3 ^{b}
Aspergillus saitoi (EC 3.4.23.18)	9.4 ^{a}	11.3 ^{a}	10.7 ^{a}	12.2 ^{a}	5.0 ^{c}	10.9 ^{a}	13.7 ^{a}	14.3 ^{a}	10.1 ^{a}	10.1 ^{a}	6.7 ^{b}	11.9 ^{a}
Control	9.4 ^{a}	8.0 ^{c}	6.7 ^{c}	6.7 ^{c}	9.4 ^{a}	8.0 ^{c}	6.7 ^{c}	6.7 ^{b}	9.4 ^{b}	8.0 ^{b}	6.7 ^{b}	6.7 ^{c}
	Stability time (min)											
Papain (EC 3.4.22.2)	6.7 ^{c}	8.6 ^{b}	6.6 ^{b}	7.7 ^{b}	6.3 ^{b}	6.4 ^{b}	7.0 ^{b}	5.0 ^{b}	6.9 ^{c}	6.6 ^{c}	6.0 ^{a}	5.5 ^{b}
Aspergillus saitoi (EC 3.4.23.18)	8.3 ^{a}	10.1 ^{a}	9.2 ^{a}	10.6 ^{a}	4.0 ^{c}	11.4 ^{a}	12.0 ^{a}	12.2 ^{a}	9.0 ^{a}	8.7 ^{a}	4.7 ^{b}	10.6 ^{a}
Control	7.7 ^{b}	6.1 ^{c}	4.7 ^{c}	4.5 ^{c}	7.7 ^{a}	6.1 ^{b}	4.7 ^{c}	4.5 ^{b}	7.7 ^{b}	6.1 ^{b}	4.7 ^{b}	4.5 ^{c}

[1] Means of Farinograph parameters of wheat flour replaced with the same fraction of treated lentil flours having the same chemical treatment and various enzymatic treatments with different letters are significantly ($P<0.05$) different according to least squares differences (LSD).

Water absorbance of treatments ranged from 56.4 to 70.0%, irrespective of chickpea and lentil chemical and enzymatic treatments. Moreover, sequential papain–acid treatment resulted in the least water absorption of legume treatment (i.e., from 56.4 to 61.9% for chickpeas and from 59.0 to 65.2% for lentils of the 5 to 20% fractional replacements). Sequential papain–acid and alkaline treatment, on the other hand, resulted in the greatest water absorption of treatments. Sequential enzymatic-alkaline treatment had no effects on modification treatment's water absorption (i.e., from 66.0 to 68.0% for chickpeas and from 67.0 to 70.0% for lentils of the 5 to 20% fractional replacements). Furthermore, the increase of disrupted chickpea and lentil flour contribution in treatments resulted in a linear increase in water absorption regardless of enzymatic and/or chemical treatment.

Water absorption results were attributed to the chemical composition and structure of modified chickpeas and lentils. The increase in water absorption would have a significant impact on strengthening the treatments of treated chickpeas and lentils. The increase in water absorption of treated samples is related to the modifications of chickpea and lentil proteins as a result of the enzymatic and chemical treatments. Water absorption can be considered as an indication of gluten network extents and maturation of gluten matrix during dough formation (Raungrusmee *et al*., 2022).

Chickpea and lentil modification also impacted the treatment's arrival time. A faster arrival time was reported with the enzymatic modification. For example, the arrival time of lentil treatments decreased from 1.8 and 3.5 min of the control to 0.7 to 1.4 min chickpea treatments and from 1.6 to 2.2 min of the lentil control to 1.0 to 1.5 min of the treated samples. Additionally, enzymatic and chemical modification of treated chickpeas and lentils resulted in a significant ($P<0.05$) increase in the development time. *Aspergillus saitoi* (EC 3.4.23.18) enzyme had the most significant impact on the development time of chickpea and lentil treatments. A similar trend is reported for the stability time of chickpea and lentil treatments. The increase in development and stability time measurements indicates a slow development of dough and a good indication of dough strength.

The changes in the arrival and development time of treated samples were attributed to the changes in the molecular size and solubility of chickpea and lentil proteins. Results are in line with Li-Hua, *et al*., (2018) findings which related the changes in departure time to the changes in molecular size of oats flour constituents including β-glucan. Furthermore, protein disruption; as a result of protein structural alteration due to the use of proteolytic enzymes could have impacted protein-starch structural arrangement (Haileslassie, 2019). Protein–starch interactions were reported to significantly affect the formation and strength of protein gel matrix during dough network (Saleh 2017).

Results were attributed to changes in the chemical structure and composition of checkpea and lentils with the greater disruption of protein using proteolytic enzymes. Flours substituted wheat flour (i.e., the greater disrupted chickpea and lentil flours) increased the ability of flours to absorb moisture during pasta making (table 1). This increase was attributed to the greater formation of smaller peptide fractions as a result of the use of peptides-derived proteolytic enzymes. Protein peptide formation is believed to play a significant part in modifying the gelatinization and gelling behavior of the modified treatments as indicated by the increase in stability during farinograph measurements. However, the extent of the effect was dependent on the peptides-derived proteolytic enzyme type with *Aspergillus saitoi* (EC 3.4.23.18) enzyme resulting in greater impacts having an average stability index of 12.95 compared to 6.9 for Papain (EC 3.4.22.2) and 7.0 for the control treatments. Results are in agreement with the findings of Xue and Ngadi (2007) and Ramos *et al.,* (2021) that the viscoelastic properties of gluten are affected by the structural properties of the gliadin and glutenin sub-fractions and the interactions between them. Similarly, the results were in accordance with Marco and Rosell (2008) and Jayaprakash *et al*., (2022) that an increase in water absorption of rice and legumes after the addition of protein isolate of smaller molecular weight.

Table 3 presents the cooking loss and water uptake of cooked pasta using flour of various fractions of chickpea and lentil flours that were sequentially treated with acid, alkaline, and with or without enzymes.

Table 3: Cooking loss and water uptake of pasta made using various fractions of treated and non-treated chickpeas and lentils flours during cooking. [1, 2]

Enzymatic Treatments	Percent Replacement	Chickpea		Lentil	
		Cooking Loss (%)	Water Uptake (%)	Cooking Loss (%)	Water Uptake (%)
Acidic and Alkaline protein extract					
No Enzymatic Treatment	0	2.5 ± 0.20 [a]	78.8 ± 5.8 [d]	2.5 ± 0.20 [a]	78.8 ± 5.8 [c]
	5	1.4 ± 0.02 [c]	82.9 ± 6.5 [d]	1.6 ± 0.08 [b]	88.6 ± 4.5 [b]
	10	1.6 ± 0.11 [b]	90.1 ± 5.3 [c]	1.4 ± 0.10 [b]	90.8 ± 1.9 [b]
	15	1.2 ± 0.09 [d]	96.0 ± 3.9 [b]	1.1 ± 0.04 [c]	94.3 ± 5.2 [b]
	20	1.2 ± 0.01 [d]	107.6 ± 3.5 [a]	1.5 ± 0.05 [b]	101.5 ± 2.0 [a]
Enzymatic Hydrolysis + Acidic and Alkaline protein extract					
Papain (EC 3.4.22.2)	0	2.5 ± 0.20 [a]	78.8 ± 5.8 [d]	2.5 ± 0.20 [a]	78.8 ± 5.8 [c]
	5	0.4 ± 0.20 [b]	116.1 ± 2.7 [c]	1.3 ± 0.04 [b]	100.3 ± 3.3 [a]
	10	0.4 ± 0.06 [b]	127.9 ± 5.2 [b]	0.9 ± 0.04 [c]	97.8 ± 4.7 [a]
	15	0.4 ± 0.02 [b]	129.9 ± 3.5 [b]	0.7 ± 0.01 [c]	92.3 ± 6.5 [b]

	20	0.4 ± 0.02 [b]	154.2 ± 5.9 [a]	0.3 ± 0.04 [d]	88.0 ± 3.7 [b]
Enzymatic Hydrolysis + Acidic and Alkaline protein extract					
Aspergillus saitoi (EC 3.4.23.18)	0	2.5 ± 0.20 [a]	78.8 ± 5.8 [d]	2.5 ± 0.20 [a]	78.8 ± 5.8 [c]
	5	1.0 ± 0.11 [b]	103.4 ± 3.9 [a]	1.2 ± 0.02 [b]	105.9 ± 1.9 [b]
	10	0.8 ± 0.04 [c]	105.9 ± 3.7 [a]	1.3 ± 0.01 [b]	108.1 ± 4.4 [b]
	15	1.1 ± 0.01 [b]	99.9 ± 5.9 [b]	1.3 ± 0.04 [b]	112.1 ± 3.0 [a]
	20	0.9 ± 0.05 [bc]	93.4 ± 4.3 [c]	0.9 ± 0.10 [c]	113.0 ± 5.2 [a]

[1] Means of cooking loss and water uptake of cooked pasta having the same chemical treatment and enzymatic treatment of various fractions replacement of disrupted chickpea and lentil flour (columns) with different letters are significantly (P<0.05) different according to least squares differences (LSD).

Modifications of the chickpea and lentil flour resulted in q significant (P<0.05) decrease in the cooking loss of pasta processing. For example, cooking loss decreased from 2.5% of the control treatment (i.e., 100% wheat flour) to 1.2 of the acid, alkaline treated chickpea samples. Similarly, cooking loss decreased (P<0.05) from 2.5% of the control sample to 0.4 and 0.3 of the enzymatically treated chickpea and lentil flours, respectively. The use of papaya (EC 3.4.22.2) enzymatic treatment was more effective in decreasing the cooking loss of cooked pasta when compared to chemical or *Aspergillus saitoi* (EC 3.4.23.18) enzymatic treatments, respectively.

Chemical treatment resulted in a significant (P<0.05) increase in water uptake of pasta during cooking. The increase in treated chickpeas and lentils resulted in a linear increase in pasta water uptake. Water uptake of cooked pasta ranged from 78.8% of the control samples to 107.6 and 101.5% of the 20% chickpea and lentil fraction treatments, respectively. Enzymatic treatment also impacted the water uptake of cooked pasta; however, varying trend was reported. More specifically, with the increase in fractions of papaya (EC 3.4.22.2) enzymatically treated chickpea; an increase of water uptake of chickpea and a decrease in water uptake of lentil treated sample were reported. Inversely, a decrease in water uptake with the increase in fractions of *Aspergillus saitoi* (EC 3.4.23.18) treated chickpea while an increase in water uptake of lentils was reported.

Water uptake of sequential chemical and enzymatic treated samples ranged from 78.8 of the control to 154.2% of 20% fractions of papaya (EC 3.4.22.2) treated chickpea and from 78.8% of the control to 113.0% of 20% of *Aspergillus saitoi* (EC 3.4.23.18) treated lentil flours. Results provide evidence of the significant role of proteins in determining the functional properties of cereals and cereal products. As chemical treatment resulted in a decrease in cooking loss with the increase in treated fractions; a steeper decrease in solids loss was reported with the enzymatic treatment.

Our results are in line with the increase in departure and arrival time of treated chickpea and lentil flours (Tables 1 and 2). During pasta cooking, it is believed that enzymatic modifications would have resulted in the formation of a strong gluten network that was able to trap water as well as solutes more efficiently. In this regatrd, Suo *et al*., (2021) reported that the presence/absence of the gluten network modulates not only solid loss in the cooking water but also starch accessibility by digestive enzymes, Saleh and Meullenet (2007) also reported that partially disrupted protein – starch network plays a significant part in binding water. The limited rupture and the formation of a strong network and thus the greater water uptake and the less leaching out of solids are in line with Derycke *et al*., (2005) findings. Table 4 presents the hardness and stickiness of cooked pasta made using 20% fractions of sequential chemical and papaya (EC 3.4.22.2) or *Aspergillus saitoi* (EC 3.4.23.18) chickpea and lentil treatments.

Table 4: Hardness (N) and stickiness (N.s) of cooked pasta made using 20% fractions of chemical and enzymatic treatments of chickpea or lentil flours. [1, 2]

Enzymatic Treatments	Chickpeas		Lentils	
	Hardness (N)	Stickiness (N.s)	Hardness (N)	Stickiness (N.s)
	Acidic protein extract			
Papain (EC 3.4.22.2)	187.0 ± 3.1 ^{b}	85.9 ± 3.1 ^{a}	32.4 ± 2.4 ^{d}	25.0 ± 1.1 ^{d}
Aspergillus saitoi (EC 3.4.23.18)	105.9 ± 2.8 ^{c}	68.4 ± 2.9 ^{ab}	97.2 ± 3.3 ^{b}	75.0 ± 3.7 ^{a}
Control	2010.6 ± 2.6^{a}	35.2 ± 1.9^{c}	2010.6 ± 2.6 ^{a}	35.2 ± 1.9 ^{c}
No Enzymatic Treatment	97.7 ± 2.4 ^{c}	60.9 ± 2.6 ^{b}	61.5 ± 2.1 ^{c}	48.1 ± 5.3 ^{b}
	Acidic and Alkaline protein extract			
Papain (EC 3.4.22.2)	81.0 ± 1.4 ^{b}	53.4 ± 1.4 ^{b}	68.9 ± 1.3 ^{b}	54.8 ± 4.4 ^{a}
Aspergillus saitoi (EC 3.4.23.18)	84.4 ± 3.7 ^{b}	83.9 ± 2.1 ^{a}	70.2 ± 1.8 ^{b}	46.1 ± 5.1 ^{ab}
Control	2010.6± 2.6 ^{a}	35.2 ± 1.9 ^{c}	2010.6 ± 1.6 ^{a}	35.2 ± 1.9 ^{b}
No Enzymatic Treatment	39.9 ± 1.6 ^{c}	30.1 ± 1.5 ^{c}	59.6 ± 2.15 ^{b}	47.1 ± 1.6 ^{a}
	Alkaline protein extract			
Papain (EC 3.4.22.2)	42.7 ± 2.2 ^{c}	30.8 ± 2.2 ^{b}	67.7 ± 6.8 ^{b}	53.3 ± 2.5 ^{a}
Aspergillus saitoi (EC 3.4.23.18)	115.8 ± 2.4 ^{b}	70.7 ± 2.7 ^{a}	62.3 ± 2.2^{b}	46.6 ± 1.1 ^{ab}
Control	2010.6 ± 2.6^{a}	35.2 ± 1.9 ^{b}	2010.6 ± 2.6^{a}	35.2 ± 1.9 ^{b}
No Enzymatic Treatment	111.7 ± 1.9 ^{b}	69.9 ± 2.0 ^{a}	64.6 ± 1.4 ^{b}	50.0 ± 1.0 ^{a}

[1] Means of cooked pasta hardness (N) and stickiness (N.s) of the same chemical and enzymatic treatments (B, D or no enzymes) of chickpea and lentil treatment with different letters are significantly (P<0.05) different according to least squares differences (LSD).

The hardness of cooked pasta ranged from 39.9 to 2010.6 N for chickpeas and from 32.4 to 2010.6 N for lentil treatments. Results indicate a significantly (P<0.05) softer cooked pasta with either chemical or sequential chemical and enzymatic treatment. For example, cooked pasta hardness decreased from 2010.6 N of the control sample to 111.7, 97.7, and 39.9 N for alkaline acid and sequential acid then alkaline treated chickpea flour samples, respectively. Similarly, cooked pasta hardness decreased from 2010.6 N of the control sample to 50.0, 48.1, and 47.1 N for similarly treated lentil flour samples, respectively. Sequential chemical and enzymatic treatments also resulted in a reduction of cooked pasta hardness; however, with a milder effect than just the chemical modification. Cooked pasta stickiness ranged from 35.2 N.s of the control sample to 85.9 N.s. for chickpeas and 75.0 N.s for lentil treatments. Sequential alkaline and papaya (EC 3.4.22.2) treatment resulted in a significantly (P<0.05) less sticky pasta for chickpea treatment with a stickiness value of 30.8; while sequential acid and papaya (EC 3.4.22.2) treatment resulted in a significantly (P<0.05) less sticky pasta for lentil treatment with a stickiness value of 25.0 N.s.

The decrease in cooked pasta hardness with chickpea and lentil flour treatments resulted in weakening the structure of starch–protein granular network

formation. A modification in the sensory properties was observed by the addition of protein smaller polypeptides as a result of the photolytic enzymes. The increased formation of polypeptides is believed to increase starch's ability to absorb moisture to its maximum extent, swell, and disrupt faster than with the non-disrupted control with minimal shear force. This justifies the strong correlation between hardness and an increase in dough water absorption and cooked pasta water uptake of treated samples. These results are in line with Fitzgerald *et al*., (2003) who indicated that protein disruption limits the formation of a protein network resulting in a greater absorption of water and softer cooked riceSharma *et al*., (2022) in the same manner reported that Pasta exhibited lower lightness and higher yellowness than the control pasta, with firmness and toughness modulated that owed to the complex interaction between potato starches and the gluten protein matrix.

Stickiness for almost all treated samples was significantly ($P<0.05$) greater than that of the control; although treated samples had significantly ($P<0.05$) less leached-out materials. These results suggest that disrupted chickpea and lentil proteins support the formation of a sticky protein-leached-out starch network, attached to the surface of cooked pasta which is responsible mostly for cooked pasta stickiness (Sharma *et al*., 2022). Table 5 presents the sensory attributes of cooked pasta made using 20% fractions of treated chickpea and lentil flour.

Table 5: Sensory attributes (i.e., overall liking, firmness, stickiness, flavor and color) of cooked pasta made using 20% fractions of Acid + Alkaline and various enzymatic treatments of disrupted chickpea and lentil flours. [1, 2]

Enzymatic Treatment	Chickpea				
	Overall liking	Firmness	Stickiness	Flavor	Color
	Acidic protein extract				
Papain (EC 3.4.22.2)	6.3 ± 0.7 [b]	7.8 ± 1.0 [b]	7.1 ± 1.0 [b]	7.5 ± 0.2 [ab]	7.9 ± 0.8 [b]
Aspergillus saitoi (EC	6.5 ± 0.7 [b]	7.5 ± 0.8 [b]	7.4 ± 0.9 [b]	7.8 ± 0.2 [a]	7.3 ± 0.8 [c]
Control	7.5 ± 0.5 [a]	8.8 ± 0.3 [a]	8.5 ± 0.4 [a]	7.3 ± 1.0 [b]	8.5 ± 0.6 [a]
No Enzymatic Treatment	4.5 ± 0.9 [c]	7.6 ± 0.9 [b]	6.3 ± 0.9 [c]	7.4 ± 0.1 [b]	7.4 ± 0.9 [c]
	Acidic and Alkaline protein extract				
Papain (EC 3.4.22.2)	5.2 ± 0.4 [b]	6.9 ± 0.2 [c]	5.6 ± 0.3 [d]	7.2 ± 0.1 [a]	7.5 ± 0.6 [b]
Aspergillus saitoi (EC	5.0 ± 0.6 [b]	7.5 ± 0.9 [b]	6.3 ± 0.8 [c]	7.4 ± 0.2 [a]	7.3 ± 1.0 [bc]
Control	7.5 ± 0.3 [a]	8.8 ± 1.0 [a]	8.5 ± 0.8 [a]	7.3 ± 0.2 [a]	8.5 ± 0.7 [a]
No Enzymatic Treatment	4.2 ± 0.1 [c]	6.5 ± 0.6 [c]	7.2 ± 0.9 [b]	7.1 ± 0.1 [a]	7.2 ± 0.8 [b]
	Alkaline protein extract				
Papain (EC 3.4.22.2)	5.1 ± 0.8 [b]	5.7 ± 0.8 [d]	6.5 ± 0.8 [c]	7.9 ± 0.9 [a]	7.1 ± 0.5 [c]
Aspergillus saitoi (EC	5.6 ± 0.8 [b]	7.7 ± 0.7 [b]	7.7 ± 0.7 [b]	7.8 ± 0.8 [a]	7.8 ± 0.7 [b]
Control	7.5 ± 0.8 [a]	8.8 ± 0.8 [a]	8.5 ± 0.8 [a]	7.3 ± 0.8 [b]	8.5 ± 0.9 [a]
No Enzymatic Treatment	4.3 ± 0.4 [c]	6.3 ± 0.2 [c]	6.4 ± 0.6 [c]	7.2 ± 0.7 [b]	7.7 ± 0.8 [b]
	Lentils				
	Acidic protein extract				
Papain (EC 3.4.22.2)	6.0 ± 0.5 [b]	7.1 ± 0.5 [b]	6.5 ± 0.8 [b]	7.9 ± 0.5 [a]	7.9 ± 0.6 [b]
Aspergillus saitoi (EC	6.2 ± 1.0 [b]	8.4 ± 0.7 [a]	6.2 ± 0.7 [bc]	7.4 ± 0.4 [b]	7.5 ± 0.8 [b]
Control	7.5 ± 0.9 [a]	8.8 ± 0.8 [a]	8.5 ± 0.6 [a]	7.3 ± 1.0 [b]	8.5 ± 0.4 [a]

No Enzymatic Treatment	5.8 ± 0.7 [b]	7.0 ± 0.7 [b]	5.8 ± 0.6 [c]	7.7 ± 0.1 [a]	7.8 ± 0.2 [b]
			Acidic and Alkaline protein extract		
Papain (EC 3.4.22.2)	6.9 ± 1.0 [b]	5.3 ± 0.8 [c]	5.2 ± 0.6 [b]	7.9 ± 0.8 [a]	8.1 ± 0.4 [a]
Aspergillus saitoi (EC	6.1 ± 0.5 [c]	6.1 ± 0.5 [b]	5.9 ± 0.7 [b]	7.7 ± 0.6 [a]	8.1 ± 0.6 [a]
Control	7.5 ± 0.8 [a]	8.8 ± 0.8 [a]	8.5 ± 0.7 [a]	7.3 ± 0.6 [b]	8.5 ± 0.7 [a]
No Enzymatic Treatment	6.1 ± 0.5 [c]	5.1 ± 0.5 [c]	5.5 ± 1.0 [b]	7.1 ± 0.4 [b]	7.8 ± 0.4 [a]
			Alkaline protein extract		
Papain (EC 3.4.22.2)	6.8 ± 0.7 [b]	7.5 ± 0.8 [c]	6.0 ± 0.6 [c]	7.5 ± 0.8 [a]	7.6 ± 0.7 [ab]
Aspergillus saitoi (EC	6.6 ± 1.0 [b]	8.1 ± 0.7 [b]	7.0 ± 0.8 [b]	6.9 ± 0.6 [a]	7.1 ± 0.7 [b]
Control	7.5 ± 0.8 [a]	8.8 ± 0.7 [a]	8.5 ± 0.9 [a]	7.3 ± 0.2 [a]	8.5 ± 1.0 [a]
No Enzymatic Treatment	5.9 ± 0.9 [c]	7.0 ± 0.7 [c]	6.1 ± 1.0 [c]	7.6 ± 0.3 [a]	7.1 ± 0.8 [b]

[1] Means of cooked pasta sensory attributes of the same chemical (acid, acid + alkaline, and alkaline) and various enzymatic treatments (B, D or no enzymes) of disrupted chickpea or lentil 20% replacement fractions with different letters are significantly (P<0.05) different according to least squares differences (LSD)

Chemical and enzymatic treatments of chickpea and lentil resulted in significantly lower (P<0.05) scores of the overall acceptance, firmness, and stickiness of cooked pasta than the control. For example, overall acceptance scores of treated samples ranged from 4.2 to 6.8 for chickpeas and from 5.8 to 6.9 for lentils compared to 7.5 for the control pasta. Similarly, the control pasta sample had a firmness score of 8.8 compared to 5.7 to 7.8 for chickpeas and from 5.1 to 8.4 for lentil-treated flours. As for stickiness, control pasta had a score of 8.5 compared to 5.6 to 7.7 for chickpeas and 5.2 to 7.0 for treated lentil flours. The lower scores of chickpea and lentil-treated samples than the control were inter-correlated with correlation confidences ranging from 0.66 (i.e., between overall and stickiness scores of the sequential chemical and enzymatic chickpea flour) to 0.98 (i.e., between the overall and color scores of the sequential chemical and enzymatic chickpea flour). On the other hand, the scores of flavor of treated chickpea and lentil samples were superior (i.e., P <0.05 for most treatments) compared to the control.

Sharma *et al*., (2022) reported that the lightness scores of starch pasta products impacted by the fractions of other ingredients used. Although the flavor of modified pasta was superior to the control, it seems that the negative impacts of chickpea and lentil modifications on cooked pasta texture and color lowered the overall acceptance score. Modification treatments of chickpea and lentil flours resulted in a greater tendency of treatments to absorb more moisture (Table 3) resulting in softer pasta. Our results are in agreement with Teterycz *et al*., (2020) on the same manner indicated a significant impact of pasta as a result of chickpea addition.

Conclusion

Chemical and enzymatic treatments of chickpea and lentil flours resulted in significant changes in pasta functional characteristics. Protein disruption as a result of modified chickpea and lentil flour is the key factor in changing cooked pasta texture properties. Fragile protein-starch network formation was believed to play a significant role in modifying pasta texture resulting in softer and stickier cooked pasta, and lowered pasta cooking loss; a significant factor in evaluating pasta quality. The findings of this study provide significant information toward enhancing pasta functionality and suggesting a reduction in cooking duration for pasta in order to achieve the desired texture.

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"تأثيرات التعديلات الأنزيمية لمستخلصات بروتين البقوليات [أي الحمص (Cicer arietinum) والعدس (Lens culinaris)] على الخصائص الوظيفية للمعكرونة"

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ملخص

بحثت هذه الدراسة في تأثير التحلل المائي الأنزيمي لمستخلصات بروتين الحمص والعدس على الخصائص الوظيفية للمعكرونة. تم استبدال دقيق القمح بنسب 0، 5، 10، 15، 20% من مستخلص البروتين المتحلل والمستخلصات غير المتحللة من دقيق العدس والحمص. يشمل علاج الدراسة علاجات إنزيمية متتابعة تليها علاجات حمضية ثم قلوية .كان للتحلل المائي الأنزيمي لمستخلصات بروتين البقوليات تأثير كبير على الخصائص الوظيفية للبقوليات. أدت زيادة التحلل المائي لبروتين البقوليات إلى زيادة خطية في امتصاص الماء بغض النظر عن التحلل المائي الأنزيمي و/أو طريقة الاستخلاص الكيميائي. أظهرت النتائج انخفاضًا في امتصاص الماء مع التحلل المائي للبروتين بينما أدى التحلل المائي الأنزيمي قبل معالجة استخلاص البروتين إلى أكبر امتصاص للماء بين المعالجات. بالإضافة إلى ذلك، أثرت علاجات الحمص والعدس على الفارينوغراف المعالج مما أدى إلى وصول أسرع مع التعديل الأنزيمي. على سبيل المثال، انخفض وقت الوصول من 138 إلى 318% للعدس ومن 123 إلى 183% للحمص، مما يشير إلى وقت أسرع لتطور العجين مما يساهم في توفير وقت تجهيز المعكرونة .انخفض فقدان المعكرونة أثناء الطهي من 2.5% في معاملة المقارنة إلى 1.2% (أي تخفيض بنسبة 208%) في الحمص المعالج بالحمض والقلوية وإلى 0.4% (أي تخفيض بنسبة 625%) في عينات العدس المعالجة. كان استخدام المعالجة الأنزيمية غراء (EC 3.4.22.2) هو الأكثر فعالية في تقليل الفقد طهي المعكرونة المطبوخة. كما انخفضت صلابة المعكرونة المطبوخة من 2010.6 ن في العينة الضابطة إلى 97.7 و39.9 و111.7 ن للحامض الحمضي المتتابع ثم للمعاملة القلوية للحمص على التوالي. تم الإبلاغ عن نتائج مماثلة للعدس مع صلابة تتراوح بين 48.1، 47.1 إلى 50.0 ن. ولذلك، فإن استخدام التعديلات الأنزيمية من شأنه أن يسهم في تحسين خصائص جودة المعكرونة.

الكلمات الدالة: المعكرونة، الخصائص الوظيفية، دقيق الحمص، دقيق العدس.

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