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Technological properties and non-enzymatic browning of white lupin protein enriched spaghetti

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Abstract

Spaghetti was prepared by replacing semolina with different amounts of lupin protein, in order to increase the protein content. A detailed investigation of the rheological properties of the dough and the cooking quality of pasta was performed in comparison to standard semolina spaghetti. Moreover, the effect of the addition of lupin protein on non-enzymatic browning was evaluated by measuring ε-furoylmethyllysine (furosine) and 5-hydroxymethyl-2-furancarboxaldehyde (HMF), which are considered useful indices of semolina quality and pasta processing conditions. Dried spaghetti fortified with 5% of lupin protein isolate has a colour and rheological features comparable with the semolina sample and also the behaviour during cooking results to be satisfactory. As far as the thermal damage is concerned, the furosine values of fortified spaghetti differ only marginally from standard pasta and the percentage lysine loss is quite small (ranging from 12.1% to 15.7%).

Keywords: Extensograph; Farinograph; Furosine; HMF; Maillard reaction; Rheological properties

1. Introduction

Consumers have a growing interest in foods containing components that may be beneficial for health, in particular for preventing cardiovascular disease (de Roos, 2004). After the US Food and Drug Administration (FDA) approval (FDA, 1999) of the health claim concerning the role of soy protein in reducing the risk of heart disease (Anderson, Johnstone, & Cook-Newell, 1995; Sirtori et al., 1998), a great variety of functional foods based on soybean proteins have been introduced on the US market with a very good consumer acceptance.

A slower growth has been observed in Europe (Keinan Boker, Peeters, Mulligan, Navarro, & Slimani, 2001), mainly due to the public concern about the use of genetically modified organisms (Baker & Burnham, 2001). For this reason, the European industry has great interest for other plant proteins to be used as functional ingredients (Frost & Sullivan, 2001).

Two recent papers have reviewed all available data on the possible role of grain legumes in the prevention of the main risk factors of cardiovascular disease (Anderson,

Abbreviations: BU, Brabender unit; DDT, dough development time; E, Energy; ESI, electrospray ionization; E_x, extensibility; FU, farinograph unit; furosine, ε-furoylmethyllysine; HMF, 5-hydroxymethyl-2-furancarboxaldehyde; LDL, low density lipoprotein; LPI-E, lupin protein isolate E; MC, maximum consistency; NEB, non-enzymatic browning; R_{50}, resistance to extension at 50 mm; S, semolina; SPE, solid phase extraction; TI, tolerance index.

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2003; Arnoldi, 2004). The genus *Lupinus* appears to be particularly promising, since recent findings have indicated that a moderate daily intake of white lupin protein leads to the reduction of total and low-density lipoprotein (LDL) cholesterol levels, when administered to an established rodent model of hyperlipidemia (Sirtori et al., 2004) and some specific protein fractions may be useful in controlling hyperglycemia (Magni et al., 2004). The same activities were confirmed also by a preliminary clinical study (Naruszewicz, 2005). In addition, very recently, a diet based on narrow-leaf lupin seeds showed hypolipidemic activity in a pig model of hypercholesterolemia (Martins et al., 2005).

White lupin has some other positive features: in particular there are no genetically modified varieties commercially available and the content of the main antinutritional components is very low (Champ, 2002; Katagiri, Ibrahim, & Tahara, 2000; Muzquiz et al., 1998; Sirtori et al., 2004).

As in the case of soybean protein the effective daily dose is 25 g, a similar amount may be envisaged also for lupin: such a large value may be achieved only if a portfolio of several lupin based food products with very satisfactory sensory characteristics is available. As pasta is a main staple food in some South European countries, such as Italy and Greece, the objective of this study was to prepare spaghetti fortified with different amounts of lupin protein and to compare the rheological properties of the dough and the cooking quality of pasta with standard semolina spaghetti.

Part of the research was dedicated to investigate non-enzymatic browning (NEB). In fact some products deriving from this reaction (Arnoldi, 2001; Ledl & Schleicher, 1990) are considered useful indices of semolina and pasta quality, in particular ε-furoylmethyllysine (furosine) (Anese, Nicoli, Massini, & Lerici, 1999; Pagani, Resmini, & Pellegrino, 1992; Resmini, Pagani, & Pellegrino, 1996) and 5-hydroxymethyl-2-furancarboxaldehyde (HMF) (Acquistucci, Bassotti, & Cubadda, 1988). Considering that grain legume proteins are much richer in lysine than wheat proteins, it may be easily foreseen that even the addition of a small percentage of these proteins may greatly increase these markers. Being the fortification of pasta with legume protein relatively unusual (Bahnassey & Khan, 1986; Dick & Youngs, 1988), so far nothing has been published on this topic, whereas some scattered information is available in the case of other foods, such as infant cereals (Guerra-Hernandez, Corzo, & Garcia-Villanova, 1999), biscuits (Resta, D'Agostina, Boschin, & Arnoldi, 2005), and bread (Ramirez-Jimenez, Garcia-Villanova, & Guerra-Hernandez, 2000).

2. Materials and methods

2.1. Materials

This work was based on a semi-industrial lupin protein isolate characterised by good emulsifying properties (LPI-E), produced by the Fraunhofer Institut für Verfahrenstechnik und Verpackung (Freising, Germany) (Wasche, Muller, & Knauf, 2001). 2D-electrophoresis coupled with HPLC-ESI-MS/MS showed that this material contains mostly β-conglutin (7S globulins) and α-conglutin (11S globulins), whereas γ-conglutin is only a minor component (Wait et al., 2005). Commercial semolina (S) was obtained from a milling industry (Allatini Flour Mills, Thessaloniki, Greece). Semolina granulation was measured by a mechanic shaker (Retac 4D, Germany) using 100 g of sample and shaking for 5 min with US Standard Testing Sieves 35, 40, 60, 80, 100, 230 (500, 425, 250, 180, 150, 63 μm, respectively). More than 93% of particle size distribution was below 425 μm. To investigate the effect of blending on product quality, blends were prepared by mixing semolina with 5%, 10%, 15%, and 20% LPI-E.

2.2. Chemicals

Furosine (purity 99.5%) was purchased from Neosystem (Alltech, Milan, Italy); HMF (purity 99%) from Sigma–Aldrich (Steinheim, Germany); HPLC-grade methanol and acetic acid from Baker (Deventer, Netherlands); HCl (37%) from Riedel-de Haën (Seelze, Germany). Water used as eluent in HPLC analysis of furosine and KCl (purity 90%) were purchased from Fluka (Milan, Italy), whereas water used in all purification procedures and for the analysis of HMF was produced with a MilliQ Water Purification System (Millipore, Billerica MA; USA). SPE cartridges High Capacity C18 Extract-Clean (bed-weight 500 mg, tube size 4 mL, particle size 50 μm, pore size 60 Å, carbon loading 17%) and 0.45 μm disposable nylon filters for HPLC eluents and samples were from Alltech (Milan, Italy). All other reagents were analytical grade from Merck (Darmstadt, Germany).

2.3. Proximate chemical analysis

The nitrogen content was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to determine the protein content; the moisture, ash, starch and total fat contents were determined according to ICC methods 110/1, 104/1, 123/1, and 136, respectively ICC, 1994a, 1994b, 1994c, 1994d. All the determinations were expressed on a dry weight basis. Each value was the average of three measurements.

2.4. Rheological characteristics of the flour (or flour blends) during mixing

2.4.1. Farinograph procedure

The mixing properties of the doughs obtained from the different semolina/LPI-E blends were examined with a Brabender farinograph (Brabender, Duisburg, Germany) according to the method of Irvine (1971). All samples were screened at 33% water absorption. The dough development time (DDT) is defined as the time (in minutes) measured after the addition of water to the point of the curve immediately before the first sign of decrease in consistency. The

maximum consistency (MC) is defined as the consistency (in FU) measured at the development time and in the middle of the curve bandwidth. The tolerance index (TI) is the decrease in consistency (in FU), which occurs 4 min after the MC.

2.4.2. Extensograph procedure

A Brabender extensograph (Brabender, Duisburg, Germany) was used for measuring the stretching properties of the blend doughs, in particular the resistance to extension and the extensibility. Doughs from the farinograph measurements were cut into two parts of 150 g each and passed through the balling and moulder unit of a Brabender extensograph. After 45 min resting in the fermentation cabinet, the dough was stretched. After this first test, the balling and moulding operations were repeated and the doughs were tested again after a further 45 min resting time. The same procedure was repeated for a third time, following the official procedure (ISO, 1988). The results were expressed as: (a) energy (E), which is the work applied for stretching the dough and is an index of the flour quality (expressed in cm^2); (b) resistance to constant deformation after 50 mm stretching (R_{50}); (c) extensibility (E_x), which is the distance travelled by the recorder paper from the moment in which the hook touches the test piece until rupture of the test piece; (d) the ratio between the last two parameters (R_{50}/E_x). The results are given in BU with a precision of 5 BU.

2.5. Formulation of spaghetti

Each semolina/LPI-E blend (1 kg) was mixed for 10 min with 350 mL tap water. The dough was processed into spaghetti (1.75 mm diameter) using an experimental press (3 kg capacity, Namad, Rome, Italy) in two replicate batches. The dough was then extruded under vacuum of 480–550 mm Hg, after mixing for another 10 min. The spaghetti was then dried in a laboratory dryer (Namad, Rome, Italy) at 60 °C for 18–20 h.

2.6. Cooking characteristics of spaghetti

The optimum cooking time (min) for each sample was determined using the AACC method 66–50 (AACC, 2000). The spaghetti were considered cooked when the white core had disappeared after having pressed the spaghetti between two Plexiglas plates. The cooked weight (g) (a measure of the degree of spaghetti hydration) of dry spaghetti sample was recorded as described by the AACC method 66–50 (AACC, 2000). Cooking loss, expressed as percent, was measured by evaporating the spaghetti cooking water to dryness in a oven at 100 °C, as described by the AACC method 66–50 (AACC, 2000). Firmness of raw and cooked spaghetti samples was measured with a Texture Analyser TA-XT2i equipped with a special shearing tooth according to the AACC method 66–50 (AACC, 2000). Results were expressed as the peak force (g) and peak energy ($g \times cm$) required while shearing one strand of spaghetti.

2.7. Colour measurements

The colour of dried spaghetti was measured with a Hunterlab colorimeter, model MiniScan XE Plus. Colour readings were expressed by Hunter values for L^*, a^* and b^*. L^* values measure black to white (0–100), a^* values measure redness when positive, and b^* values measure yellowness when positive. Spaghetti strands were disposed in an adapted stand that allowed a flat surface to be formed. Reported values are the means of independent triplicate determinations.

2.8. Furosine determination

In a screw-capped Pyrex-vial, an aliquot of sample corresponding to about 500 mg of protein was hydrolysed with 8 mL 8 N HCl. The closed vials were sealed under vacuum, kept at 110 °C for 23 h, and then filtered on a paper filter. The chromatographic determination of furosine was performed by RP-HPLC following a literature procedure for the determination in milk (Resmini, Pellegrino, & Battelli, 1990) with small modifications. A portion of the filtrate was applied to an Extra-Clean C18 cartridge (Alltech), pre-wetted with 5 mL of methanol and 10 mL of water. The sample (0.5 mL) was added, the displaced liquid was discarded, and furosine was eluted with 3 mL of 3 N HCl. The chromatographic analyses were performed on a C_8 Alltech furosine-dedicated column (250 mm × 4.6 mm). Conditions: eluent A 0.4% acetic acid in water, eluent B 0.3% KCl in eluent A (w/v); gradient 0% B for 13.5 min, 0% B to 50% B in 7 min, then 50% B for 2 min; flow rate: 1.2 mL/min; inject volume 20 μL. UV detection wavelength 280 nm. Furosine was eluted at retention time (t_R) 22–25 min. The quantification was performed through the external standard method and the results were expressed as mg/100 g protein. Each value is the mean of at least three determinations.

2.9. HMF determination

Each sample (100 mg) was hydrolysed with 1 mL 0.3 N oxalic acid at 100 °C for 3 h. After rapid cooling in ice, 0.5 mL diluted trichloroacetic acid (40% w/v) was added. The sample was centrifuged at 12,063g for 12 min, and then filtered through a 0.45 mm filter before injecting in HPLC. The chromatographic determination of HMF was performed by RP-HPLC, following a procedure originally applied to milk (Morales, Romero, & Jimenez-Perez, 1992). The chromatographic analyses were performed on a C_{18} Spherisorb ODS-2 column, (250 × 4.6 mm, 5 μm, Merck). Conditions: mobile phase 100% sodium acetate buffer 0.08 mM adjusted to pH 3.6 with acetic acid; flow rate 1 mL/min; injection volume 20 μL; detector wavelength 284 nm. HMF was eluted at t_R 7–9 min. The quantification was performed by the external standard method

using a calibration curve and the results were expressed as mg/100 g protein. Each value is the average of at least three determinations.

3. Results and discussion

3.1. Physicochemical properties of semolina and blends

Proximate chemical analysis of semolina, and semolina/LPI-E mixtures are shown in Table 1. As expected, all blends showed higher protein and ash content and lower moisture, fat and starch content than semolina. The protein content of the blend containing 20% LPI-E increased by more than 100%. The semolina used had a gluten content of 30.2%, gluten index 79, and falling number 503 s.

3.2. Dough rheological properties

The farinograph mixing curve at 33% absorption provides information on rheological properties of pasta dough (Table 2). The development time is the time between the first addition of water and the moment in which the dough reaches the greatest torque. During this mixing phase, water hydrates the flour components and the dough is developed. In general, stronger gluten samples give longer mixing curves (see the semolina sample in Table 2). By adding LPI-E, the dough development time and maximum consistency decrease: this weakening effect may be probably the result of the dilution of the gluten structure of semolina by lupin protein. The tolerance index is relatively small for all samples, indicating that the gluten of the semolina sample was elastic and extensible.

The extensograms showed that, increasing the LPI-E percentage from 0% to 20%, the doughs were less extensible as indicated by higher ratios of R_{50}/E_x, while the area under the curve (i.e. the energy required to break the strength of dough) increased substantially by adding up to 5% LPI-E and thereon decreases, still remaining above the semolina value (Table 2). This indicates that the doughs of the blends are still strong and elastic. However, when the amount of LPI-E was increased up to 50% (data not shown), the dough became very weak and the stability and development time decreased as well as the extensibility and the resistance.

3.3. Spaghetti cooking quality

The cooking quality is the most important feature of pasta that encompasses the following characteristics: (a) the weight of cooked pasta indicating the water uptake during cooking; (b) the cooking loss, related to solid leaching during cooking and considered as an indicator of the overall cooking performance; (c) the texture of the cooked product, which indicates firmness and resilience.

The results of spaghetti cooking performance in terms of optimal cooking time, cooked weight and cooking losses are presented in Table 3. The optimal spaghetti cooking time is independent of the LPI-E percentage and within the normal range of semolina spaghetti. Water uptake during cooking is responsible for the texture of cooked spaghetti. In semolina spaghetti the ideal expected cooked weight is about three times larger than the dry weight (Dick & Youngs, 1988). This is the case of semolina spaghetti and the 5% blend. On the contrary, the addition of higher amounts of LPI-E produces a significant decrease in the cooked weight, which becomes dramatic when the incorporation reaches 15% and 20%. A similar behaviour was found by Bahnassey and Khan (1986) who supplemented spaghetti with different legume flours and protein concentrates.

Cooking loss values lower than 7–8% are expected for semolina spaghetti (Dick & Youngs, 1988). Table 3 demonstrates that increasing LPI-E percentages are responsible of increased cooking losses and that the incorporation of

Table 1
Proximate chemical composition[a] of blends of semolina (S) and lupin protein isolate E (LPI-E)

Blends S/LPI-E	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Starch (%)
100/0	13.48	0.88	12.02	2.16	73.92
95/5	12.43	1.12	15.42	1.75	71.22
90/10	11.86	1.27	18.73	1.62	63.60
85/15	11.64	1.35	21.86	1.46	61.41
80/20	11.90	1.41	25.41	1.34	57.35

[a] Expressed on dry weight basis. Values are the averages of three analyses.

Table 2
Effect of lupin protein isolate E (LPI-E) supplementation of semolina (S) on the rheological characteristics of the doughs

Blends S/LPI-E	Farinograph			Extensograph (135 min)	
	DDT (min)	TI (F.U.)	MC (F.U.)	E (cm^2)	R_{50}/E_x
100/0	10.0	15	520	89	6.50
95/5	5.5	20	500	173	6.92
90/10	4.5	20	500	128	9.70
85/15	4.0	30	470	102	9.97
80/20	3.5	40	470	98	10.30

The results of the farinograph measurements are given as dough development time (DDT), tolerance index (TI) and maximum consistency (MC). The results of extensograph measurements are given as energy, which is the area under the extensogram, and the ratio between the resistance to deformation (R_{50}) and the extensibility (E_x).

Table 3
Cooking characteristics of spaghetti samples containing lupin protein isolate E (LPI-E), measured by using AACC method 66–50 (AACC, 2000)

Spaghetti composition S/LPI-E	Optimal cooking time (min)	Cooked weight (g)	Cooking loss (%)
100/0	3.9	33.20	7.50
95/5	3.8	32.04	8.71
90/10	4.1	28.10	14.47
85/15	3.8	25.10	15.31
80/20	4.1	23.61	16.93

The values are the mean of three measurements.

more than 5% LPI-E results in an unacceptable cooking loss (which becomes 17% for the 20% blend).

Of course these results agree with the cooked weight decreasing in the same samples. Rayas-Duarte, Mock, and Sattarlee (1996) similarly reported an increase in cooking loss vs. increasing substitution of lupin flour for semolina. The main technological role of durum wheat gluten is the formation of an internal network that holds the pasta components together. The addition of lupin protein isolate seems to dilute the gluten strength and weaken the overall structure of the spaghetti.

The firmness of cooked pasta and the surface condition of pasta after cooking (which is known to be related to the loss of matter in the cooking water and to the tendency to stickiness) are the two major characteristics of pasta cooking quality. Differences in cooking quality in terms of firmness are primarily attributed to gluten. Gluten of high quality and strength, as assessed by the physical tests (farinograph, extensograph), produces spaghetti of optimum quality. That is why high-gluten spaghetti are internally firmer than low-gluten spaghetti after cooking. Spaghetti with 5% and 10% LPI-E retain firmness and the force required to break increases (Figs. 1 and 2). The firmness [force (*g*)] of dried or cooked spaghetti increases with increasing LPI-E content (Table 4). A high degree of mechanical strength of the raw product is desirable in spaghetti in order to minimise breakage during handling (Hollinger, 1963).

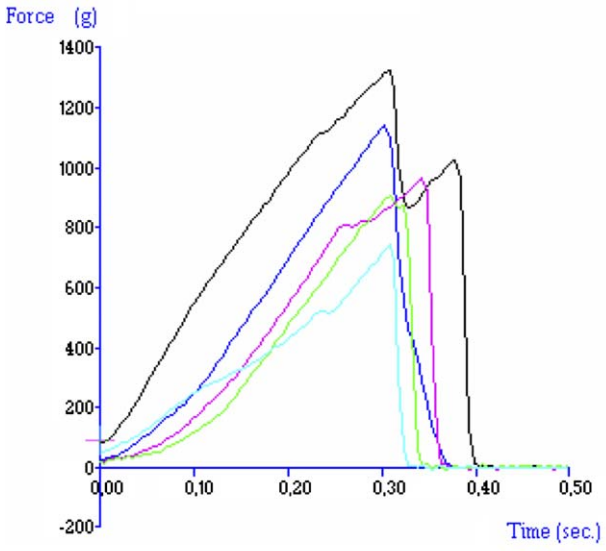

Fig. 1. Plots of firmness (*g*) vs. time (s) of uncooked lupin protein-enriched spaghetti measured with a Texture Analyser (TA-XT2i), according to AACC method 66–50 (AACC, 2000).

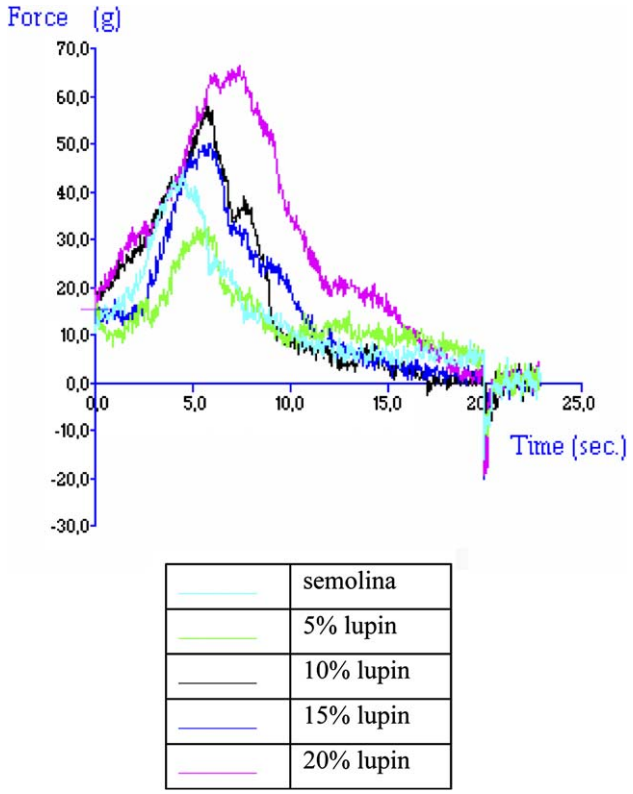

Fig. 2. Plots of firmness (*g*) vs. time (s) of cooked lupin protein-enriched spaghetti measured with a Texture Analyser (TA-XT2i), according to AACC method 66–50 (AACC, 2000).

Table 4
Firmness of uncooked and cooked spaghetti fortified with lupin protein isolate E (LPI-E) measured with a Texture Analyser (TA-XT2i), according to AACC method 66–50 (AACC, 2000)

Spaghetti composition S/LPI-E	Uncooked		Cooked	
	Peak force (*g*)	Area (*g* cm)	Peak force (*g*)	Area (*g* cm)
100/0	749.8	6.02	45.7	13.57
95/5	914.3	5.92	44.4	13.80
90/10	1020.7	6.95	58.3	18.05
85/15	1148.8	9.19	57.3	19.19
80/20	972.7	8.14	66.9	28.60

The results are the mean of five independent measurements.

3.4. Colour characteristics

Colour measurements were performed only on dry spaghetti (Table 5). By replacing LPI-E for semolina, the colour became darker (lower L^* values) and redder (higher a^* values), whereas yellowness (b^* values) remained practically constant. Yellowness and brightness are correlated both to the pigment concentration and to enzymatic reactions, whereas redness is generally related with the development of NEB (Oliver, Blakeney, & Allen, 1993).

Table 5
Colour of spaghetti enriched with lupin protein isolate E (LPI-E) measured with a Hunterlab colorimeter

Spaghetti composition S/LPI-E	a^*	b^*	L^*	$100-L^*$
100/0	1.97	19.81	60.10	39.90
95/5	3.48	20.31	58.48	41.52
90/10	4.79	20.29	52.65	47.35
85/15	5.39	20.11	50.22	49.78
80/20	6.28	19.32	47.98	52.02

Colour readings are expressed by Hunter values for a^*, b^*, L^* and $100-L^*$. The results are the mean of three independent analyses.

3.5. Non-enzymatic browning

Two molecular markers of NEB were selected for this investigation: furosine and HMF. Furosine is produced by acid hydrolysis of the Amadori compounds formed by the Maillard reaction between reducing sugars and proteins (Resmini & Pellegrino, 1991), whereas HMF is a well known intermediate of the 1,2-enolisation route of the decomposition of the Amadori rearrangement product (Ledl & Schleicher, 1990). Both are considered useful markers of the thermal treatment in different food products that are correlated with the parameters of the industrial processes or are useful quality indexes during storage (Ramirez-Jimenez, Guerra-Hernandez, & Garcia-Villanova, 2003).

The level of furosine and HMF in LPI-E was respectively 10.24 ± 0.66 μg/100 g protein and 2.15 ± 0.12 μg/100 g protein: considering the high lysine percentage in this food ingredient (47.5 g/g protein), these values are low and had been obtained through careful optimisation of the production parameters, especially during spray-drying (D'Agostina et al., 2006). The same markers in the semolina used for the preparation of spaghetti were 27.4 ± 3.42 μg/g protein and 17.4 ± 0.40 μg/100 g protein, respectively.

Since the low water content of pasta dough is particularly favourable for NEB (Resmini et al., 1996), in order to sort out the effects of the addition of a lysine-rich protein such as lupin, drying was conducted in relatively mild conditions, at 60 °C for 18–20 h (Acquistucci, 2000; Pagani et al., 1992). At the end of the drying process, the furosine value in the semolina spaghetti became 193.9 ± 0.21 μg/100 g protein and the HMF value 147.4 ± 0.71 μg/100 g protein.

The effects of the addition of LPI-E on NEB markers is shown in Fig. 3. Indeed, the most intriguing feature of this figure is the unexpected behaviour of HMF, which decreases instead of increasing. Two possible explanations may be proposed: the first is that HMF is very sensitive to the decreasing amount of reducing sugars in the samples, the second that part of the HMF already present in raw semolina reacted with the ε-amino group of lupin protein lysine to give some other by-products.

On the contrary, the level of furosine increases proportionally vs. LPI-E addition up to 10%: over this value the dependence becomes asymptotic, indicating a saturation

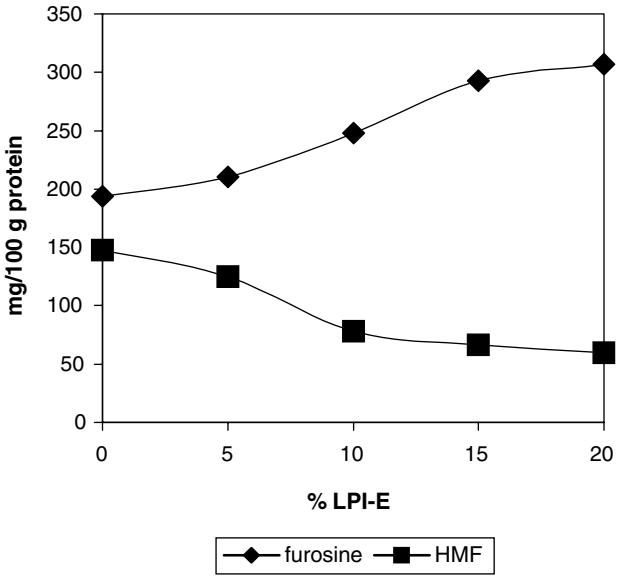

Fig. 3. Dependence of furosine and HMF in respect to percentage of added lupin protein isolate E in uncooked protein-enriched spaghetti.

phenomenon, probably related to the limited availability of reducing sugars for further lysine glycosylation. As a consequence even after the addition of 20% LPI-E, furosine exceeds only slightly 300 mg/100 g protein.

A main sensory consequence of NEB is food browning, which may be measured by the colour indexes a^* and $100-L^*$. As both indexes have been used to assess the nutritional quality of pasta and the consumer acceptance (Acquistucci, 2000), we decided to look for a possible correlation between them and furosine (Fig. 4). Indeed furosine and the colour indexes showed a very good linear correlation, although they measure different phenomena, as furosine is a marker of the early stages of NEB, whereas the colour indexes are markers of the advanced stage of NEB. In fact, the correlation equation between $100-L^*$ and furosine was $Y = 19.999 + 10.438\ X$ (where $Y = 100-L^*$ and $X =$ furosine), with a correlation coefficient $R^2 = 0.9731$, while the correlation equation between furosine and a^* was $Z = -3.859 + 3.294\ X$ (where $Z = a^*$ and $X =$ furosine), with only a slightly worse correlation coefficient $R^2 = 0.9301$.

In order to estimate the nutritional damage produced during processing a widely used indicator is unavailable lysine (or lysine loss). There are several chemical tests for the estimation of reactive lysine, which are very useful indicators of biologically available lysine in raw materials (Acquistucci, 2000). However, in processed foods some errors have been found in the final calculation because, after early NEB, many lysine units can react with reducing sugars to give deoxyketosyl derivatives, which are sometime included in the reactive lysine estimation, although they are biologically unavailable (Acquistucci, 2000). Therefore in processed foods the furosine method is considered one of the most suitable (Hurrel & Carpenter, 1981). Blocked lysine was calculated from furosine apply-

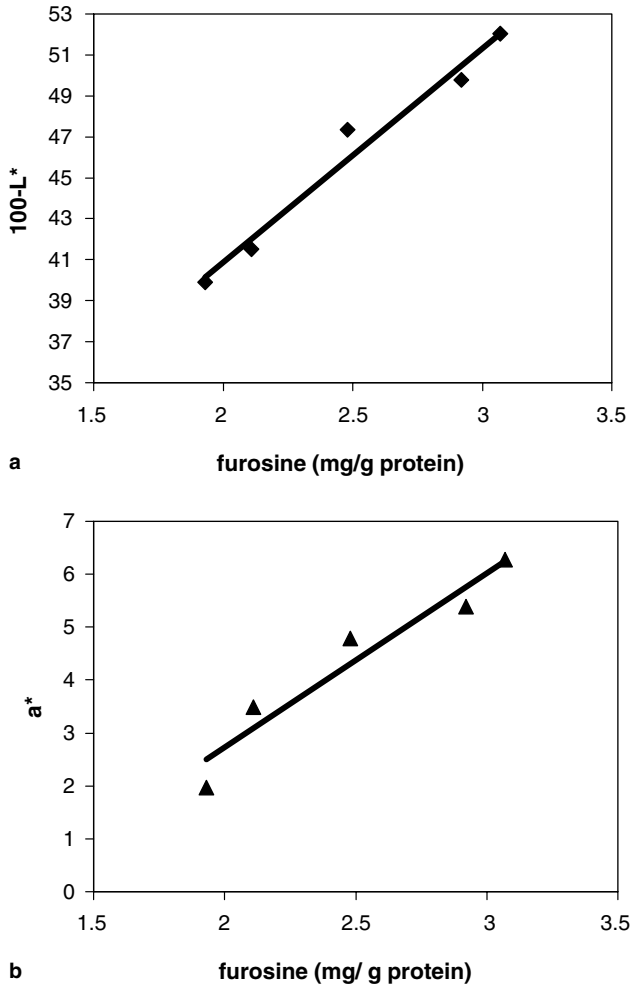

Fig. 4. Dependence of colour indexes $100-L^*$ (a) and a^* (b) vs. furosine value of uncooked spaghetti fortified with different lupin protein isolate E (LPI-E) percentages.

Table 6
Furosine, total lysine, available lysine and percentage lysine loss in spaghetti samples enriched with lupin protein isolate E (LPI-E)

Spaghetti composition S/LPI-E	Furosine (mg/g protein)	Lysine (mg/g protein)	Available lysine (mg/g protein)	Total loss (%)
100/0	1.93	23.2	20.4	12.1
95/5	2.11	24.4	21.4	12.4
90/10	2.48	25.6	22.1	13.7
85/15	2.92	26.8	22.6	15.7
80/20	3.07	28.1	24.0	15.7

The furosine values are the mean of three analyses. Available lysine was calculated by the equation proposed by Erbersdobler and Dehn-Muller (1989).

ing the mathematical expression proposed by Erbersdobler and Dehn-Muller (1989).

The obtained values are reported in Table 6. As a consequence of the LPI-E addition, blocked lysine, which was 2.8 mg/g protein in normal semolina spaghetti, increased progressively and reached the value of 4.4 mg/g protein, when the percentage became 20%. This may appear a great increase, however, it is important to underline that the main outcome of the addition of LPI-E is a consistent increase in the lysine content, which from 23.2 mg/g in semolina spaghetti becomes 28.1 mg/g protein for the addition of 20% LPI-E. Taking this in consideration, the final consequence is that the available lysine content in the samples containing LPI-E is higher than in semolina spaghetti and the percentage lysine loss remains rather small (ranging from 12.1% to 15.7%). These values are perfectly in line with those reported in literature for traditional pasta submitted to mild processing (Acquistucci, 2000; Pagani et al., 1992).

In conclusion the fortification of spaghetti with up to 5% LPI-E permits to obtain a functional food product endowed by an acceptable colour, by satisfactory standard parameters defining the cooking quality, and by good nutritional characteristics. In the countries were pasta is a staple food, the daily consumption of a normal serving of these spaghetti (dry weight = 80–100 g) would correspond to a lupin protein intake of 4–5 g, about one fifth of the dose that each hypercholesterolemic patient should consume for reducing the cardiovascular risk.

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