



Quality and nutritional properties of pasta-products enriched with immature wheat grain.

Journal:	<i>International Journal Of Food Sciences & Nutrition</i>
Manuscript ID:	Draft
Manuscript Type:	Research Paper
Date Submitted by the Author:	n/a
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Keywords:	Immature wheat grain, FOS, Pasta, Glycemic index, furosine

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2 **Quality and nutritional properties of pasta-products enriched with immature wheat grain.**

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15 Key words: Immature wheat grain, FOS, pasta, glycemic index, furosine

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4 **1 ABSTRACT**

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6 2 The study evaluated nutritional and sensory properties of pasta enriched with 30% Immature Wheat Grain
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8 3 (IWG), a natural source of fructo-oligosaccharides (FOS).

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10 4 Nutritional value, glycemic index (GI), colour and cooking quality of pasta were assessed in comparison
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12 5 with commercial inulin enriched and 100% whole wheat pastas. IWG integration induced deep changes in
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14 6 color, without negatively affecting pasta cooking quality, and promoted nutritional quality by increasing
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16 7 fiber content; IWG pasta presented a remarkable leaching of FOS in cooking water, thus providing only 1
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18 8 g of FOS per serving. IWG pastas showed a GI of 67 (dried) and 79 (fresh), not significantly different
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20 9 from commercial pasta products. IWG can be considered an interesting ingredients to obtain functional
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22 10 products “naturally enriched” in FOS and fiber. Results about FOS leaching suggest that, in dealing with
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24 11 functional effects, the actual prebiotic content should be carefully considered on food “as eaten”.
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1 INTRODUCTION

2 The development of food products that provide benefits beyond their traditional nutritional values has
3 focused academic, industrial and public interest. In the last decade nutritional research has provided
4 scientific evidence-based nutritional recommendations for the prevention of degenerative disease, which
5 include low glycemic load and high fiber intake (Kendall et al.. 2010; Miller et al.. 2009) and health
6 authorities worldwide recommend an increase of cereal intake, especially wholegrain, which is an
7 important source of dietary fiber and other bioactive compounds (Fardet 2010).

8 Among fiber fractions with recognized functional properties, FOS and inulin, non-digestible fructose
9 polymers, are also classified as “prebiotics” for their capability of “selectively stimulate growth and/or
10 activity of one or a limited number of microbial genus(era)/ species in the gut microbiota that confer(s)
11 health benefits to the host” (Roberfroid et al. 2010). Many studies in animal models and in man showed
12 that FOS and inulin tend to reduce the risk of colon carcinogenesis (Pool-Zobel et al. 2007), to enhance
13 mineral absorption (Scholz-Ahrens et al. 2007), to modulate lipid metabolism (Brighenti 2007) and the
14 secretion of gastrointestinal peptides involved in appetite (Parnell et al. 2009; Cani et al. 2009) or in
15 gastric emptying regulation (Russo F et al. 2011). FOS occurs naturally in food plants including cereals
16 such as wheat (Ritsema et al. 2003). In cereals kernels FOS are accumulated at higher level during the
17 grain filling, specifically in the physiological stage called “milky phase”, occurring 2 to 3 weeks after
18 flowering; thereafter FOS content per kernel rapidly decrease (D’Egidio et al. 1997; Ritsema et al. 2003).
19 Corradini et al.. (2003) reported that FOS present in immature wheat grains (IWG) kernels are branched
20 molecules having both β 2-1 and β 2-6 fructosyl-fructose linkages and a low degree of polymerization.
21 Compared to wheat at complete ripeness, IWG contains also less starch content with a reduced
22 availability to enzymatic digestion (Casiraghi et al.. 2006), and more fibre and soluble sugar (Iametti et
23 al.. 2006). IWG has a total protein content similar to wheat at late ripening stage, with a predominance of
24 albumins with an equilibrated aminoacidic composition (Mujoo et al. 2003) and do not contain gliadins
25 (Iametti et al.. 2006). SDS-PAGE and immuno-blotting studies indicated that no immunoreactive gluten
26 material is present in IWG before 13 days after anthesis (Iametti et al.. 2006). IWG thus appears as an
27 innovative raw material with interesting functional characteristics not only as a natural FOS source, but
28 also as an ingredient with other potential nutritional properties. However, the incapacity of IWG to form a
29 gluten network (D’Egidio et al., 1998) would recommend its use in mixtures with conventional wheat

1 flour or semolina to produce basic foods such as pasta, bread, biscuits, while a direct use could be
2 proposed for soup or baby foods. Previous works demonstrated that a 30% addition can be considered the
3 maximum level of IWG whole-meal consistent with a conventional pasta-making process (Pagani et al.,
4 2003). Moreover recent in vitro results substantiate the potential of IWG as a prebiotic ingredient and in
5 vivo data suggest an effect of IWG enriched biscuits on gastric emptying and satiety (Casiraghi et al.
6 2011). In this study IWG enriched pasta (fresh and dried) was evaluated, in comparison with commercial
7 similar pasta products, for nutritional, technological and sensorial quality. Furthermore, postprandial
8 glucose response was determined to evaluate the Glycemic index of IWG pastas.

9 **MATERIAL AND METHODS:**

10 IWG flour was obtained from kernels of wheat cultivar Duilio harvested two weeks after anthesis. These
11 immature grains were dried at 35°C for 24-30 h to reduce native humidity from about 65% to 13% and
12 milled. For pasta production a mixture at 30% integration was prepared by mixing commercial semolina
13 (cultivar Duilio) and IWG. Pasta samples (spaghetti shape, $\varnothing = 1.65$ mm) (IWGd) were produced in a
14 single batch on a pilot plant, using a low temperature ($T_{max} 50^{\circ}C$) drying diagram. Fresh pasta (IWGf),
15 tagliatelle shape, was produced on a pilot plant from Pavan Industry and then underwent a pasteurization
16 treatment (95°C for 1 min) before packaging, to roughly simulate a commercial process. For IWGd and
17 IWGf optimal cooking time was evaluated every 30 s during cooking according to Approved Method 66-
18 50 (AACC 2001). Two commercial dried pasta samples were used for comparison: a dietetic product
19 tailored for diabetic diet, enriched with inulin and resistant starch (INU), and a 100% whole wheat pasta
20 (WW).

21 In the raw pasta samples soluble, insoluble, and total dietary fiber were quantified by the enzymatic
22 gravimetric procedure (Prosky 1994). Analysis of moisture, ashes, lipids, and proteins was made by
23 AACC standard methods (AACC 2001). FOS content was evaluated, on raw and cooked pasta samples
24 by ion-exchange chromatography after water extraction and enzymatic hydrolysis in accordance with
25 Prosky and Hoebregs (1999). Carbohydrates were evaluated, on raw and cooked samples, as simple
26 sugars (Zygmunt et al. 1982), total and resistant starch (Brighenti et al. 1998).

27 Spaghetti color was determined by Minolta CR-300 Chromameter and results were evaluated on the CIE
28 1976 $L^*a^*b^*$ space. Furthermore, the occurrence of furosine was tested by HPLC method in accordance
29 with Resmini et al. (1990). Evaluation of spaghetti cooking quality was performed, only on dried pasta

1 samples, by sensory evaluation as proposed by D'Egidio et al. (1996) scoring firmness, stickiness and
2 bulkiness of pasta samples from 10 (= dislike extremely) to 100 (= like extremely) for each attribute. The
3 product was judged acceptable, good or excellent if the overall score was ≤ 70 , ≤ 80 or > 80 respectively.

4 2.1 Glycemic Index Test:

5 Glycemic index evaluation was conducted in accordance with the standard protocol ISO 26642:2010. Ten
6 healthy subjects volunteered for the study, five men and five women, age 25.8 ± 0.5 (mean \pm SEM) years,
7 BMI 21.7 ± 0.8 kg/m², basal glucose 4.2 ± 0.1 mmol/L. Written informed consent was obtained from
8 each subject. The study was approved by the Research Ethics Committee of the University of Milan.
9 Seven different test meals were given, in random order on separate mornings, at breakfast, after an
10 overnight fast, served with 500 mL of water or un-sugared tea. Meals contained 50 g of available
11 carbohydrates, either from pasta (IWGd, IWGf, INU and WW served with 10g of olive oil) or from 50 g
12 of glucose (three repeated test meals). Meals were given between 0800 and 0830 and were eaten within
13 10 min. Blood glucose was assayed using an automatic analyzer (YSI Stat 2300, Yellow Springs, OH,
14 USA).

15 2.2 Calculations and Data Analysis:

16 The glycaemic index (GI) was calculated from the 120 min incremental post-prandial area for blood
17 glucose, ignoring area beneath the baseline, by using glucose as a reference (GI = 100). Glycemic Load
18 ($GI/100 \times$ portion carbohydrate content) was calculated considering portion of 70 g and 120g for dried and
19 fresh pasta respectively as indicated by Italian LARN (1996). In addition, in accordance with Rosén et al..
20 (2009), the course of post-prandial glycaemia was analyzed by calculation a glycaemic profile (GP). GP
21 was calculated by the ratio between the time (min) during which the blood glucose was above fasting
22 concentration and the incremental peak value (mM) of blood glucose for each subject and test meal. In
23 vivo data were expressed as means \pm SEM. These results were submitted to Repeated Measures Analysis
24 of Variance; if significant effects ($p < 0.05$) were found by ANOVA, the differences between products
25 were checked by Tuckey HSD pos-hoc test (Stat Soft for Windows, release4.5, Statsoft Inc; Tulsa; USA).

26

27 **RESULTS AND DISCUSSION:**

28 The aspect of dried pasta, especially its color, is an important characteristic for product acceptability by
29 the consumers. Addition of IWG is associated with deep changes in the color of dried pasta: IWGd, and

1 to a lesser extent, IWGf pasta showed a brown index higher than that observed in INU pasta but lower
2 than that evaluated in the commercial WW pasta (Figure 1). It is likely that the darkening partially arises
3 from the bran content in IWG, and partially is the result of non enzymatic browning related to Maillard
4 reactions during the drying process (Pagani et al. 2010). In fact, our previous works comparing IWG with
5 mature wheat (D'Egidio et al. 2007) showed higher α -amylase activity, lysine and soluble sugars contents
6 in IWG suggesting a high susceptibility of this ingredient to the Maillard reaction. To evaluate the extent
7 of this reaction in tested samples, the occurrence of furosine (\square -fructosyl-lysine), derived from the
8 decomposition of Amadori compounds at high temperatures (Pagani 2010) was studied. IWGd pasta
9 presents a roughly 30% lower furosine content if compared with WW (Figure 2); conversely, lower levels
10 of this water-soluble furanic compound were assessed in INU and, obviously, in IWGf pasta, a product
11 not subjected to drying. Compared to literature data (Pagani et al. 2010; Zardetto et al. 2003), furosine
12 levels evaluated in IWGd are indicative of a mild heat-damage and suggestive of a 30% loss of available
13 lysine (Resmini et al. 1990). Stated the more balanced aminoacid composition and, in particular, the
14 higher lysine levels of IWG (4.46 mg/100 g protein) with respect to mature durum wheat (2.93 mg/100 g
15 protein) (Nardi et al., 2003), it is likely that IWG pastas could improve the daily intake of this essential
16 aminoacid.

17 IWG integration does not negatively affect pasta cooking quality, as shown by the sensory assessment
18 results (Table 1); firmness, stickiness and bulkiness appear very similar in IWGd and in WW and
19 resulted in a total score (63 and 65) higher than that observed in INU pasta (56), which was judged more
20 sticky and less firm than IWGd. Thus the presence of fructo-oligosaccharides in pasta seems to influence
21 its structure differently: in IWGd firmness resulted higher and stickiness lower than in INU, suggesting
22 that the pasta structure remains more compact. This might be due to a different interference between
23 inulin and FOS, probably related to their different degree of polymerization, with the protein strands,
24 resulting in weaker starch-protein binding in the case of longer molecules (Manno et al. 2009).

25 The nutritional composition and energy content of tested pasta samples are reported in Table 2.

26 The limited integration (30%) of durum wheat semolina with IWG wholemeal promotes interesting
27 nutritional characteristics in pasta samples: IWG enriched products showed a fiber content very similar
28 to that evaluated in WW pasta, with slightly higher levels of the metabolic active soluble fraction

1 (Raninen et al. 2011). IWG pastas showed a FOS content of 2.2-2.9 % d.b, very similar to that assessed
2 in commercial inulin enriched pasta .
3 Comparing FOS content in raw and cooked pasta, is evident a leaching of FOS in cooking water ranging
4 from 30 to 60% of the amount evaluated in uncooked samples of IWGd and IWGf respectively (Figure 3)
5 and probably due to the solubility of fructo-oligosaccarides in water; actually, a similar behavior, , was
6 found also for commercial pasta with inulin. In IWGf the leaching of FOS during cooking is not reduced
7 as expected from the short cooking time of fresh pasta; on the contrary, it appears higher than in dry
8 pasta. This result could be related to the differences in the structure of fresh and dried pasta (Petitot et
9 al.2009) and, as suggested by Tudorica et al. (2002), to the fact that fiber integration affects the strength
10 of the gluten network more negatively in fresh pasta, leading to a product characterized by a weaker
11 structure.
12 Moreover, these results about FOS leaching appear noteworthy to be considered in dealing with
13 functional food enriched with prebiotics : in the light of a functional effect, the actual prebiotic content
14 should be carefully considered on food “as eaten”. Stated the loss of FOS in cooking water, a portion of
15 IWG enriched pasta (70g dried pasta and 120 g fresh pasta) will provide 1 g of FOS. Unfortunately there
16 are very few and often contrasting published data regarding FOS or Inulin intakes in European
17 population: considering a mean daily 5g intake of these fructo-oligosaccarides (Coussement et al. 1999;
18 Dunn et al. 2011), a portion of enriched pasta can increase the estimated FOS intakes by a roughly 20%.
19 The post-prandial glucose response (figure 4) was similar among pasta meals, showing a peak value at 30
20 min and a return to baseline values within 2 hours with no significant differences in blood glucose
21 concentration at any given time points. GP, defined as the duration for incremental post-prandial blood
22 glucose response divided with the blood glucose incremental peak, is a useful tool for evaluation of post-
23 prandial glycaemia. Products characterized by high GP, indicative of a lower glucose peak and a less
24 pronounced hypoglycaemia and thus of a favorable postprandial glycaemic response, are more prone to
25 induce benefits on second-meal glucose tolerance (Rosén et al.. 2009). As judged from their higher GPs,
26 it could be argued that IWGd, IWGf and WW pastas are characterized by a more beneficial glucose
27 regulation than I. The calculated GI values of IWG pastas resulted slightly higher but not significantly
28 different than those evaluated for commercial products. Pasta is a popular carbohydrate-based food with a
29 low glycemic response. A continuous protein matrix which entraps starch granules and/or limits/retards

1 starch hydrolysis by α -amylase is thought to be an important factor in explaining the slow digestion of
2 starch in pasta and, consequently, its low glycemic impact (Fardet et al. 1998). International GI tables
3 show that the glycemic index for different type of pasta remains in the low (0–55) to medium (56–69)
4 range (Foster-Powell et al. 2002). IWGd has a medium GI which, together with its proximate
5 composition, confers to a portion (70g) of this pasta a GL of 14, very similar to that evaluated for INU
6 and WW pasta. Conversely, IWGf pasta presents a high GI and GL. This trait could be related, at least in
7 part, to a more porous and open protein/starch network and completely gelatinized starch granules
8 assessed in fresh sheeted pasta in comparison with extruded/dried pasta, characterized by a more compact
9 structure presenting ungelatinised starch granules (Pagani et al. 1989; Petitot et al. 2009). Moreover, the
10 inclusion of insoluble fibre may disrupt the protein matrix, giving rise to a highly porous structure in
11 which starch granules become more susceptible to enzyme degradation (Tudorica et al. 2002) and thus to
12 an increased GI (Kristensen et al. 2010)

13 **Conclusions:**

14 There is growing evidence that whole-grain cereal products protect against the development of chronic
15 diseases (Kelly et al. 2007). These protective effects of wholemeal cereals have been attributed to their
16 different components with biological activity such as dietary fiber, vitamins and other substances with
17 antioxidant properties (Fardet A. 2010). The availability of functional cereal products naturally enriched in
18 FOS and fiber could play an important role in increasing fiber intakes, promoting gastrointestinal health
19 and reducing the glycemic load of the diet. In this contest, a product like pasta may be a suitable strategy,
20 since it represent an appealing food consumed worldwide that combine cheapness, ease of preparation,
21 and long shelf life. In addition the use of functional pasta, like IWG enriched, would represent a minimal
22 change in the diet population which habitually consume pasta and it could be maintained for a long
23 period. Our results, however, highlight the complexity of the development of a product like pasta, which
24 is subjected to further cooking treatment, in order to maintain sensory and nutritional qualities, in addition
25 to its functional potential. Thus, in order to optimize these traits, and, overall, to limits FOS leaching
26 during cooking, still more technological research will be necessary, trying to control the modification of
27 food structure induced by IWG enrichment, which is of great importance also on the ability to modulate
28 the glycaemic response of pasta. Finally further intervention studies in humans have to be planned to
29 sustain the health metabolic effects of IWG enriched pastas when included in a daily diet.

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Declaration of interest:
The authors declare no conflict of interest.

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1 Table 1 Pasta cooking quality by sensory assessment

	Firmness	Stickiness	Bulkiness	Total score
IWGd	60	60	70	63
INU	20	88	60	56
WW	60	65	70	65

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Table 2 Nutritional composition of experimental and commercial pasta before cooking (g/100 g db; mean±SD)

	Protein	Fat	Total Starch (RS)*	Sugars	FOS	Total Dietary Fiber (soluble;insoluble)	Energy kcal (kJ)
IWGd	12.30±0.40	2.14± 0.03	70.29±1.3 (1.1)	2,87±0.01	2.18±0.24	6.70±0.39 (1.60;5.10)	358 (1496)
IWGf	11,91±0.56	2.28±0.19	69.21±0.8 (1.89)	3.17±0.01	2.90±0.18	7.57±0.41 (2.52;5.05)	359 (1502)
INU	14.68±0.91	1.85±0.01	66.08±1.5 (4.5)	1.66±0.01	2.14±0.19	9.5±0.88 (1.41;7.09)	339 (1420)
WW	13.45±0.31	2.18±0.03	71.22±0.7 (2.7)	1.63±0.01	0.72±0.11	6.91±0.29 (1.39;5.52)	367 (1534)

* (RS)= Resistant Starch

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1 **Figure legends:**

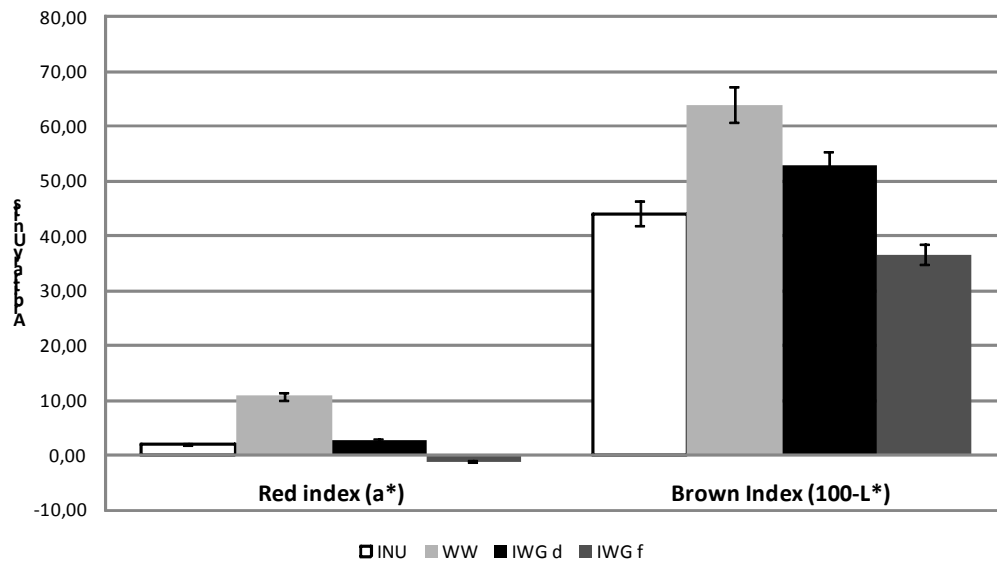
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3 **Figure 1:** Red and Brawn Indices of experimental and commercial pastas (mean \pm SD). Red index is expressed as the CIE 1976 a* coordinate; Brawn index is expressed as the CIE 1976 (100-L*).

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6 **Figure 2:** Furosin levels in experimental and commercial pastas (mean \pm SD)

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8 **Figure 3:** Fos content in a portion of raw (70g dried pasta; 120 g tagliatelle) and cooked pastas.

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10 **Figure 4:** Mean blood glucose concentration increments in healthy volunteers (n=10) following ingestion of glucose and different pasta meals and pastas' Glycemic Index, Glycemic Load and Glycemic Profile (table in insert; mean \pm sem). Points with different superscript letters are significant different within the same experimental time (p<0.05)

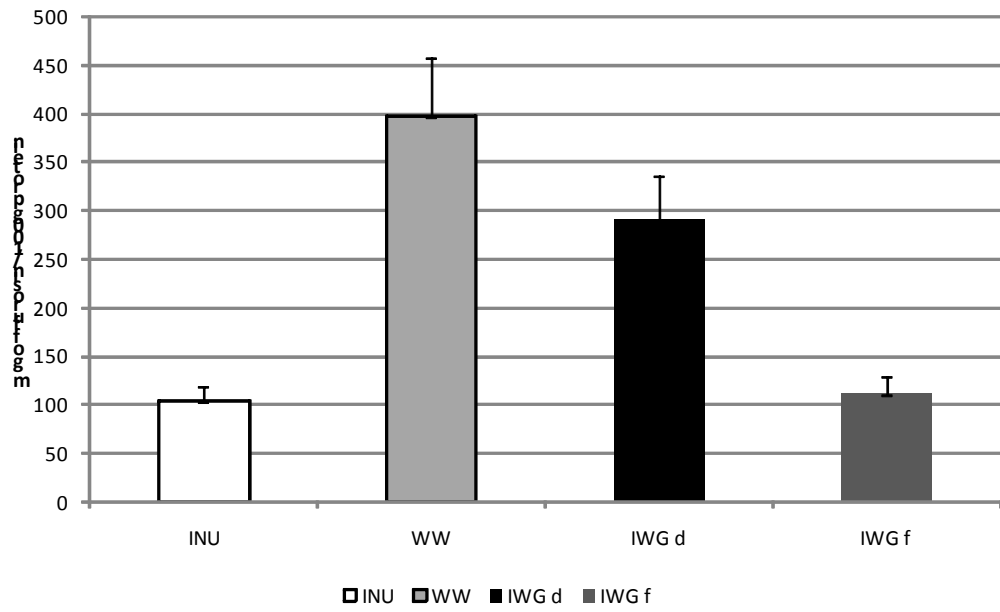
Figure 1

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Figure 2:



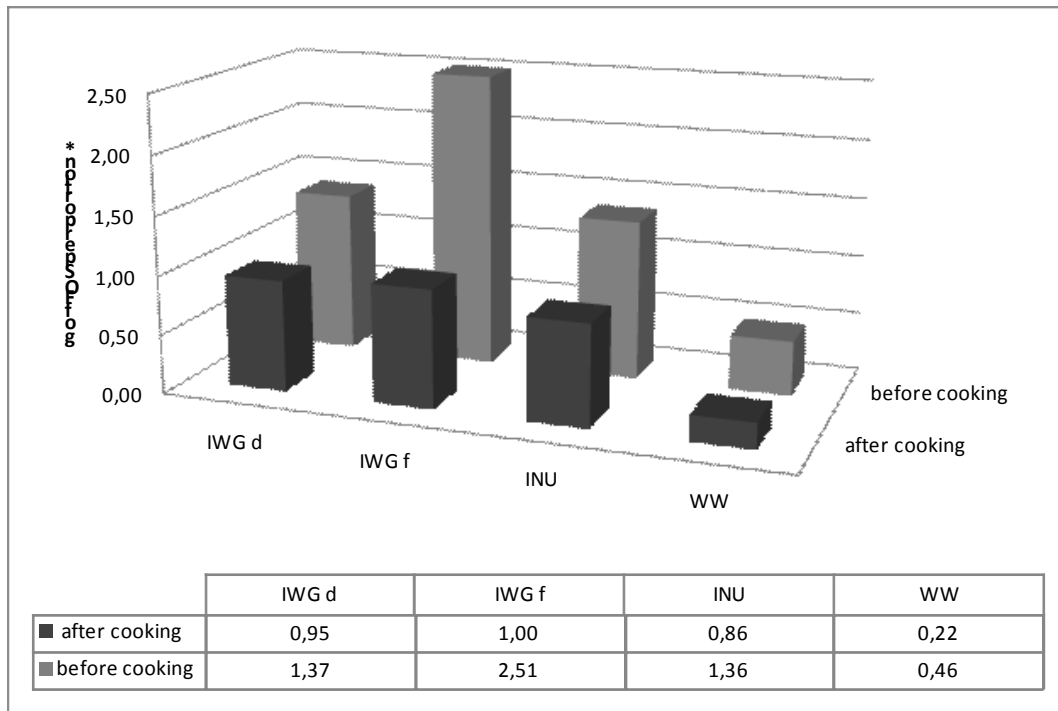
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Figure 3:



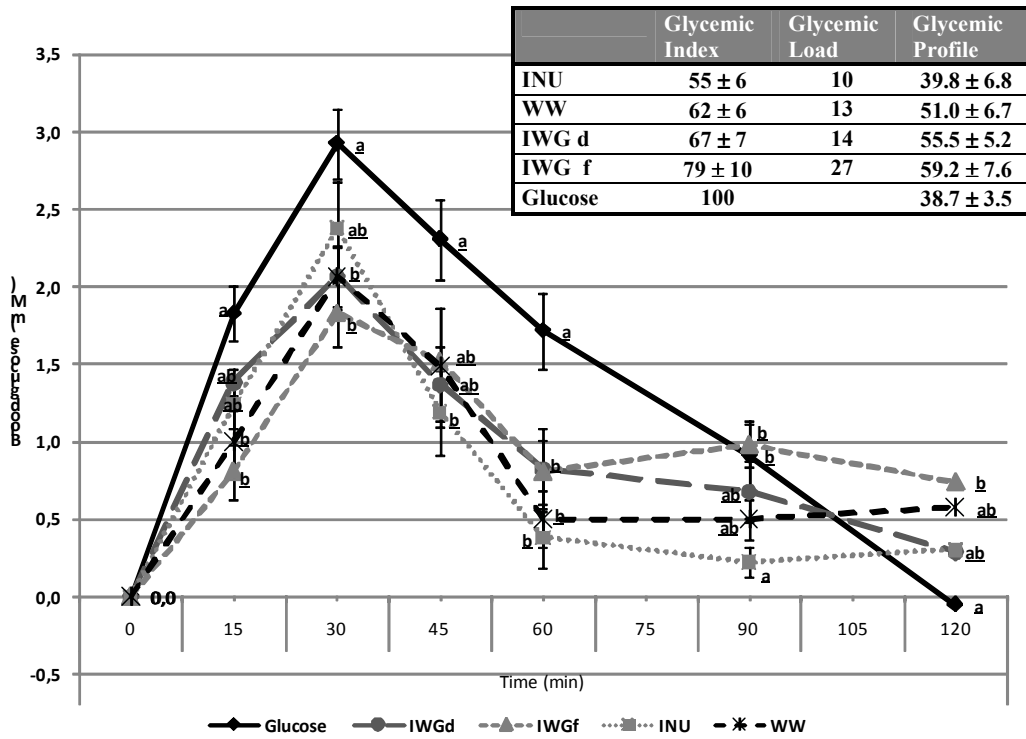
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(*): IWGf= 120 g; IWGd= 70 g

1 Figure 4:

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