Quality of spelt pasta enriched with eggs and identification of eggs using 13C MAS NMR spectroscopy


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Abstract
This paper deals with the characteristics of spelt pasta enriched with eggs. Eggs were added to spelt farina in the quantity of 0, 124 or 248 g/kg (equivalent to 0, 3 or 6 eggs, respectively). Post-hoc Tukey’s HSD test at 95% confidence limit showed significant differences between various samples. Relatively low coefficients of variation have been obtained for each applied assay (1.25–12.42%), which confirmed the high accuracy measurements and statistically significant results. Standard score analysis is applied for accessing the contribution of eggs’ content to spelt pasta quality. Maximum scores regarding quality (0.89) and chemical characteristics (0.70) have been obtained for 6 eggs spelt pasta formulation. It is also shown that the presence of eggs in pasta can be clearly confirmed by 13C MAS NMR spectroscopy. Simultaneous increase in area of peak positioned at 29.5 and 176 ppm is directly associated with the increase in the content of added eggs in the corresponding samples. Pertinent data showed positive contribution of eggs to the spelt pasta and also that NMR spectrum can be used in the egg quantity control.

Keywords: eggs, 13C MAS NMR, spelt pasta, quality.

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The dynamic way of life has the tendency to simplify and decrease in preparing healthy, quick, cheap, safe organic meals, and spelt pasta is meeting these goals. Pasta can be stored a long time without deterioration in flavor, odor and usability, and it doesn’t lose quality like bread. Pasta is also characterized by good digestion. It is a significant source of proteins with relatively satisfactory composition of essential amino acids [1]. Eggs added to pasta contribute to better mechanical properties and quality of the product and also increase the nutritive and biological value of the product, which is reflected in the increase of lysine and ω-3 fatty acids and natural sources of lecithin. By consumption of products enriched with eggs human meets recommended needs [2–5].

Spelt wheat has shown potential in various food applications, including bread, pasta, breakfast cereal and other products of altered nutritional characteristics compared to conventional wheat products. It has very high protein content and even 30 to 60% higher concentration of mineral elements Fe, Zn, Cu, Mg and P compared to Triticum aestivum [6–9]. Spelt pasta is produced without additives, food colours and it doesn’t contain genetically modified material, thus meets the requirements of an organic product. Organic food can be rated from satisfactory to good, because it contains much higher levels of nutrients [1,10].

Cross-polarization (CP) has been established as a method which provides significant enhancement in sensitivity of 13C MAS NMR spectrum of solid samples, allowing characterization of molecular structures present in the food samples in their native forms [11]. The change in 13C CP/MAS NMR peak intensity has been related to a change in molecular mobility, with higher 13C CP/MAS NMR peak intensities being due to a decrease in segmental mobility resulting in more efficient cross-polarization [12]. On the other hand, for molecular segments with long aliphatic chains (such as lipids) undergoing fast and isotropic reorientations, dipolar interactions are averaged to zero and therefore CP MAS is incapable to observe such kind of structures. Application of direct polarization (DP) 13C MAS NMR spectroscopy provides opportunity to detect both mobile and rigid structures together, also representing more quantitatively reliable method.

The aim of this study is to investigate the influence of eggs quantity on spelt pasta quality as well as the possibility of the identification of eggs by 13C MAS NMR spectroscopy via variation of quantity of lipid and protein component originated from added eggs in pasta samples.
EXPERIMENTAL

Material

In this experimental investigation the following materials were used for pasta production:

– whole meal spelt flour grown in the year 2012 in Serbia is characterized by protein, starch, sugar, lipids and cellulose content of: 15.6, 56.6, 2.20, 2.5 and 2.4% d.m (Table 1).

– liquid eggs concerning hygiene and microbiology were correct and were taken from a local food market.

Methods

Preparation of pasta

Pasta was made using the device “La Parmigiana D45” MAC 60. In a paddle mixer the whole meal moisture was adjusted to 31.5% by adding water or water and liquid eggs. Liquid eggs were first mixed into a homogeneous mass. Based on whole meal flour, applied quantity of eggs was 0, 124 or 248 g/kg (equivalent to 0, 3 or 6 eggs, respectively). Hydrated whole meal entered an extrusion worm which moved the loose dough forward and simultaneously compresses it into a homogeneous plastic mass prior to extrusion through a die with 1.4 mm diameter used for spaghetti. Mixing time was 15 min [13]. Raw pasta was dried in a cabinet drier about 12 h at controlled temperature that did not exceed 45 °C and humidity round 70% until pasta reached the moisture around 12.5%, followed by cooling to 25 °C, for 4 h and then stored at room temperature in sealed containers. Drying conditions and air flow were stringently controlled to avoid the creating of a discontinuity in the moisture gradient between the interior and exterior of spaghetti.

Pasta quality

Quality of pasta was evaluated in terms of cooking characteristics (water uptake, volume increase and cooking loss). Stickiness was sensory evaluated by numeric scores 0–10 by a panel of trained panellists. High scores were allocated to pasta with smooth, non-sticky surface [13].

Pasta texture – Texture analysis

Textural properties of cooked pasta were measured with Texture analyzer TA.HD plus (Stable Micro System, U.K.) equipped with a 5-kg load cell. The preparation procedure was the same for all tested samples (duration of cooking, time period between cooking and testing). Hardness and adhesiveness of cooked pasta were measured using a 36 mm cylinder probe (P/36R) (instrument settings were as follows, mode: measure force in compression, pre-test speed: 2.0 mm s⁻¹; test-speed: 1 mm s⁻¹; post-test speed: 10 mm s⁻¹; strain: 75%; trigger force: 5 g). The maximum force correlates to the hardness of the sample. Adhesiveness is calculated from the negative areas of the plots. Toughness (area under the force/time curve) was measured using the Warner–Bratzler shear blade (type HDP/BS) and the following settings: pre-test speed: 2 mm s⁻¹; test-speed: 2 mm s⁻¹; post-test speed: 10 mm s⁻¹; distance: 15.00 mm; trigger force: 10 g. The tests were performed on 10 replicates per batch. The two spaghetti strands were held close together and positioned centrally under the probe during testing.

Pasta colour

Pasta colour (L*- lightness, b*- share of yellow colour if positive value and blue colour if negative value, C*-differences in colouration) was measured by objectively colourimeter Chroma meter (CR-400, Konica, Minolta, Japan) and was determined according to the procedure described by Filipović et al. [5].

Chemical analyses

Basic chemical analyses: protein, starch, sugars, lipids, cellulose and sugars of pasta spelt were defined according to approved AOAC method cited by Kaluderski and Filipović [13]. Nitrogen was determined by Kjeldahl method (979.09) [14] and converted to protein using factor of 5.75.

$^{13}$C MAS NMR analyses

The $^{13}$C MAS NMR spectrum was recorded at 100.627 MHz using a Bruker MSL 400 NMR spectrometer Tecmag console upgraded (Houston, TX, USA), operating at room temperature. Samples of spelt pasta

Table 1. Chemical characteristics of whole meal spelt flour and pasta with eggs, component content, % d.m.; values with the same letter are not statistically different at the p < 0.05 level (Turkey’s HSD test)

<table>
<thead>
<tr>
<th>Component</th>
<th>Whole meal spelt flour</th>
<th>Quantity of eggs with pasta</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Protein</td>
<td>15.6±1.8 a</td>
<td>15.80±0.37 a</td>
<td>17.14±0.10 b</td>
</tr>
<tr>
<td>Starch</td>
<td>56.6±2.3 a</td>
<td>56.44±3.08 a</td>
<td>55.86±4.93 a</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.4±0.1 a</td>
<td>2.00±0.46 a</td>
<td>1.96±0.74 a</td>
</tr>
<tr>
<td>Sugars</td>
<td>2.2±0.8 a</td>
<td>2.38±0.18 a</td>
<td>2.50±0.13 a</td>
</tr>
<tr>
<td>Lipids</td>
<td>2.5±0.2 a</td>
<td>2.70±0.07 a</td>
<td>3.54±0.19 b</td>
</tr>
<tr>
<td>SS</td>
<td>–</td>
<td>0.24</td>
<td>0.58</td>
</tr>
</tbody>
</table>

60
were packed into a 7 mm zirconium rotor and spun at 4 kH. The $^{13}$C DP MAS NMR spectrum was obtained with high power decoupling during acquisition and repetition time of 30 s. The accumulation of 2048 scans was done to obtain a satisfactory signal-to-noise ratio. The Kel-F rotor end caps background signal was removed by subtracting of each of DP spectrum from spectrum of empty rotor recorded under same condition. All chemical shifts are expressed relative to tetramethylsilane (TMS) using adamantane peak observed at 29.5 ppm.

Statistical analyses

Descriptive statistical analyses for all the obtained results were expressed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) has been utilized to show relations between applied assays. Principal component analysis (PCA), used as pattern recognition technique, has been applied within assay descriptors to characterize and differentiate various analysed samples. The evaluation of one-way ANOVA and PCA analyses of the obtained results were performed using StatSoft Statistica 10.0® software. Applied methodology was the same as described before [5].

Determination of normalized standard scores (SS)

Standard scores is one of the most widely used technique to compare various characteristics of complex food samples determined using multiple measurements, where samples are ranked based on the ratio of raw data and extreme values of the measurement used. Since the units and the scale of the data from various nutritive, textural and colour characteristics measuring methods are different, the data in each data set should be transformed into normalized scores, dimensionless quantity derived by subtracting the minimum value from the raw data, and divided by the subtract of maximum and minimum value, according to following equations:

$$\bar{x}_i = \frac{\max_j x_{ij} - x_i}{\max_j x_{ij} - \min_j x_{ij}}, \quad \forall i$$

in case of “the higher, the better” criteria, or

$$\bar{x}_i = \frac{\max_j x_{ij} - x_i}{\max_j x_{ij} - \min_j x_{ij}}, \quad \forall i$$

in case of “the lower, the better” criteria, where $x_i$ represents the raw data. The normalized scores of a sample for different measurements when averaged give a single unitless value termed as SS, which is a specific combination of data from different measuring methods with no unit limitation. This approach also enables the ease of employing some others set of pasta formulations to this elaboration.

Standard scores for different samples investigated in this article were calculated and the result has been shown in Tables 1 and 2.

RESULTS AND DISCUSSION

Quality of Spelt pasta with eggs

ANOVA test shows statistically significant differences ($p < 0.05$ level, 95% confidence limit) in protein content among the values of samples with 0 eggs and pastas with 3 and 6 eggs, Table 1. Egg proteins in pasta are increasing its biological value and quantity of essential amino acids of the product. Addition of eggs in pasta also results in statistically significant difference in starch content, while there are no statistically significant differences in the values of cellulose and sugar content. As expected, analysis of variance indicates the statistically significant differences in lipid content in Spelt pasta with eggs.

Table 2. Quality of pasta with eggs; values with the same letter are not statistically different at the $p < 0.05$ level (Turkey’s HSD test)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quantity of eggs</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Quality of cooking pasta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water uptake, g</td>
<td>4.10±0.15$a$</td>
<td>4.01±0.21$a$</td>
</tr>
<tr>
<td>Volume increase, $\alpha$/ %</td>
<td>2.50±0.15$a$</td>
<td>2.40±0.30$a$</td>
</tr>
<tr>
<td>Cooking loss, $R$/ % d.m.</td>
<td>3.90±0.15$b$</td>
<td>3.62±0.24$^{ab}$</td>
</tr>
<tr>
<td>Pasta texture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness, g</td>
<td>3117.48±119.8$b$</td>
<td>3253.28±280.4$a$</td>
</tr>
<tr>
<td>Adhesiveness, g s</td>
<td>2.02±0.6$a$</td>
<td>0.37±0.22$b$</td>
</tr>
<tr>
<td>Work of shear – toughness, g s</td>
<td>13.18±0.62$b$</td>
<td>19.31±3.31$^b$</td>
</tr>
<tr>
<td>Color of pasta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L^*$ Brightness</td>
<td>70.15±1.19$a$</td>
<td>70.77±0.30$^{ab}$</td>
</tr>
<tr>
<td>Difference in tone (colors)</td>
<td>15.46±0.07$a$</td>
<td>16.25±0.19$^b$</td>
</tr>
<tr>
<td>Share of yellow color, $b^*$</td>
<td>14.96±0.16$a$</td>
<td>15.77±0.19$^b$</td>
</tr>
<tr>
<td>SS</td>
<td>0.01</td>
<td>0.63</td>
</tr>
</tbody>
</table>
samples of spelt pasta with 0, 3 and 6 eggs. This indicates that the addition of eggs, significantly affects the lipid content in the pasta and is highly appreciated as a source of monounsaturated fatty acids and ω-3 fatty acids that are necessary for normal functioning of the human organism [3].

In the of prevention of noninfectious, chronic diseases such as obesity, diabetes and cardiovascular disease, the nutrition experts recommend normal and balanced fat intake through diet. Total fat should be from 15 to 30%, saturated fatty acids (SFA) < 10%, polyunsaturated fatty acids (PUFAs) from 6 to 10% and trans fatty acids MK < 1%. The human body needs very small amounts of essential fatty acids that must enter by food and requirements for ω-3 fatty acids are even smaller. Consummation of pasta with eggs, introduces to the body ω-3 fatty acids which are necessary for the normal functioning of the human organism. According to Hayes [15], one egg contains 0.68% of ω-3 fatty acids necessary for daily human needs. Thus consumer satisfies 0.2 and 0.4% of daily needs of ω-3 fatty acids, by consummation of 100 g spelt pasta with 3 and 6 eggs, respectively.

Related to pasta quality, the addition of 6 eggs statistically significantly increases pasta water absorption in comparison to the values of the samples with 0 and 3 eggs (Table 2). Volume increase is ability of starch to swell and this parameter indicates that there were no statistically significant differences between pastas with eggs (3 and 6) and pasta without eggs. Cooking loss is one of parameter of the cooked pasta quality. Cooking loss has been decreased with addition of eggs, which indicates that protein and fatty substances from eggs reinforce and strengthen the gluten structure of the product.

The texture and colour of pasta are important quality characteristics of product and all contribute to the sensor properties of product, which are very important for consumers. Based on the analysis of the quality of the spelt pasta with 0, 3 and 6 eggs, the ANOVA test showed that the addition of 6 eggs statistically significantly increased the hardness of the pasta with 0 or 3 eggs (Table 2). Hardness of pastas is increased with the quantity of added eggs due to the positive influence of egg proteins and lipids on the gluten matrix. Similarity to sensory evaluation, analysis of variance showed that the addition of eggs (3 and 6) also statistically significantly reduces the adhesiveness of pasta and have a positive impact on the properties of the chewing. ANOVA test showed that the addition of 3 eggs statistically significantly increased the toughness of the pasta with 0 eggs (Table 2). Eggs lipids and phospholipids stabilize the three-dimensional structure of gluten. Our results are similar to results which were obtained by Raina et al. [16], who claimed that the mean value for toughness of raw pasta increased with increasing the protein level.

Eggs are also improving yellow color and taste of pasta while whole meal flour causes darker color of the finished product. Adding 6 eggs improves the brightness of whole meal spelt pasta (Table 2). Addition of eggs (3 to 6) contributes to statistically significant differences in share of yellow color and increases difference in tone. In all pasta samples the positive values of yellow color (b*) are registered. As expected, share of yellow color (b*) increases with increasing content of eggs. This parameter indicates the presence of natural pigment in egg, but it can’t quantify the egg quantity. These pigments are also recognized as natural antioxidants that positively contribute to free radicals elimination and are also anticarcinogenic agents [3].

SS is a numerical, unitless scale value, which has not consistent agreement with any measuring methods. Although it is a relative index and may not represent a specific property of different samples, previous experience with other samples [5] proved that SS provides a reasonably accurate rank of spelt pasta. In this article, standard scores are calculated for chemical properties and overall obtained data were presented in Tables 1 and 2. Standard score above 0.5 stands for the high standard in observed characteristics. Spelt pasta properties with SS above 0.5 showed more competitive qualities compared to other pastas.

Using the standard score analysis and revealing the standard scores of different spelt pasta formulations cannot only realize their position regarding other formulations, but also have a reference for developing strategies for improving their characteristics. The best scores for chemical properties and overall quality are obtained for pasta with more eggs added.

Analyzing of pasta samples by $^{13}$C MAS NMR spectroscopy

In Fig. 1 is presented the spectrum of pasta samples with different number of eggs added (0, 3 and 6), affecting incensement of protein and lipid content in the pasta samples. Line assignments in the spectrum could be divided in two region: first one belonging to starch component (60–105 ppm), and second one associated to lipid and protein component (14–40 ppm aliphatic carbons, ∼130 ppm aromatic and unsaturated carbons, and ∼175 ppm carbonyl carbons) [17,18]. The whole meal spelt flour (Table 1) used in this experiment contained about 56.6% d.m. of starch and 15.6% d.m. of protein. Other ingredients include 2.5% of lipids, 2.2% of sugars and 2.4% of cellulose. According to the chemical analysis of pasta samples given in Table 1, protein component dominated over lipids (average 17.1% of proteins compared to 3.64% of lipids). Presence of lipids significantly complicated NMR peak resolving in obtained spectrum. However, good resolved
sharp peaks (line width at half-height is less than 100 Hz) that are embedded on the relative broad resonance belonging to protein component represent strong evidence of the lipid component presence into the pasta samples. According to $^{13}$C NMR chemical shift assignment for lipids previously reported [19,20], peaks between 14–40 ppm represent saturated carbons in all fatty acid chains [18], whereas shifts observed around 130 ppm are characteristic for unsaturated carbons. The carbonyl carbons (ester and fatty acids) appeared at high field around 173 and 180 ppm, respectively. The glycerol external C$_{1',3'}$ and central C$_{2'}$ carbons regularly are observed at 62 and 69.5 ppm, respectively, but in the case of our pasta samples, they are completely overlapped by polysaccharide starch component. Therefore, as the most sensitive for this analysis, was selected peaks at 29.5 ppm in the chemical shift region of aliphatic carbons, and peak positioned around 176 ppm for the chemical shift of carbonyl carbons representing mutual contribution from fatty acids and amino acids as the result of egg addition (Fig. 1).

The values of areas for selected peaks determined from $^{13}$C MAS NMR spectrum of pasta samples with 0, 3 and 6 added eggs are represented in Table 3. Increase in peak area of signal positioned at 176 and 29.5 ppm (approximately 0.2 to 0.3 % for each egg added in the pasta sample), could be directly correlated with cumulative contribution of protein and lipid component originated from eggs addition in pasta samples. Although, discrimination between lipid and protein part could not be clearly determined from NMR spectrum presented in this work, based on the increase of peak area at selected position in the $^{13}$C MAS NMR spectrum of pasta samples could clearly confirm presence of egg component in given amount.

By increasing the eggs quantity from 0 to 6, the peak areas at 176 and 29.5 ppm are also increased, and the protein and lipid content could be calculated as follows:

- **Protein content** = \((8.54\pm1.28) + (1.69\pm0.25)\times\text{Area of peak } 176 \text{ ppm} (r^2 = 0.990, p < 0.05)\)
- **Lipid content** = \((-3.71\pm0.22) + (1.47\pm0.04)\times\text{Area of peak } 176 \text{ ppm} (r^2 = 0.999, p < 0.05)\)

**Table 3. Results of NMR analysis of pasta with eggs samples presented in the Figure 1 (peak area, %); peak areas are expressed as percentage of the total spectrum area**

<table>
<thead>
<tr>
<th>Line position, ppm</th>
<th>Quantity of eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>176</td>
<td>4.36  4.97  5.72</td>
</tr>
<tr>
<td>29.5</td>
<td>3.96  4.87  5.84</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

Based on data of investigation of quantity eggs influence on spelt pasta quality and the possibility of egg identification by $^{13}$C MAS NMR spectrum it can be concluded:

- Applied standard score analysis revealed the rank of each sample in comparison to other samples, regarding its chemical characteristics and overall quality. The best scores are calculated for pasta with more eggs added, and the best standard scores for chemical properties and overall quality (0.70 and 0.89, respectively) is experienced with 6 eggs, thus pointing at the strategies for improving spelt pasta characteristics.

- Egg lipids positively affect the pasta texture, and increase hardness (for 4 and 31%), reduce adhesiveness (for 82 and 94%) and increase toughness (for 46 and 8%) of the cooked pastas.

- Spelt pasta with eggs (3 and 6) contributes to the positive balance of essential amino acids and $\omega$3 fatty...
acids in human organism, with modified biologically and nutritive properties.

Besides significant overlapping between peaks originated from lipid and protein component originated from addition of egg component inside spelt pasta samples, $^{13}$C MAS NMR spectrum enables their mutual confirmation in order to correlate amount of egg component present in particular sample.

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REFERENCES


IZVOD

KIVALIET TESTENINE OD SPELTE SA JAJIMA I IDENTIFIKACIJA JAJA $^{13}$C MAS NMR SPEKTROSKOPIJOM


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(Naučni rad)

Dinamičan način življenja ima za tendenciju da se pojednostavi i vremenski skraćuje, a vrijednost njegovih učinaka na zdravlje obično ne se mijenja za velikotrajne vremenske periodove. U ovom radu se istražuje uticaj jaja na kvalitet i strukturne karakteristike testenine osnovanih na spelte.

Jajni melanž je dodavan u količini od 0, 124 i 248 g/kg krupice što odgovara količini od 0, 3 i 6 jaja. Postoje statistički značajne razlike između različitih uzoraka. Relativno niski koeficijenti varijacije, koji su dobijeni za svako od posmatranih mjerilo (1,25–12,42%) ukazuju na visoku tačnost mjerila i statističku značajnost rezultata. Primjerena analiza standardne ocene („standard score analysis”), radi potpunijeg sagledavanja uticaja količine jaja na kvalitet testenine od spelte. Najveće ocene („score”) za kvalitet (0,89) i za hemijske karakteristike testenine (0,70), dobijene su za formulacije testenine od spelte sa 6 jaja. Lipidi jaja pozitivno utiču na teksturu testenine, povećavaju tvrđinu (od 4 do 31%), smanjuju lepljivost (za 82 i 94%) i povećavaju žilavost (za 46 i 8%) kuvane testenine. Jaja dobavlja šećer, senzorne osobine integralne kuvane testenine od spelte, doprinose povećanju svetloće i udela žutog tona. Takođe je pokazano da se prisustvo jaja u testenini može jasno potvrditi pomoću $^{13}$C MAS NMR spektroskopijskih mjer. Rezultati pokazuju da testenina sa jajima od spelte ima dobri tehnološki kvalitet, a da se u kontroli kvalitete može koristiti NMR analiza za dokaživanje prisustva jaja.

Ključne reči: Jaja • $^{13}$C MAS NMR • Testenina • Kvalitet