# Effect of addition of various sugars on fermentation process of Croatian indigenous dry sausage Kulenova seka

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Scientific paper

## Abstract

*The aim of this study was to investigate the influence of addition of various sugars (glucose, sucrose, lactose, maltodextrin) on fermentation process of Kulenova seka made according to the traditional recipe. Physical-chemical properties of meat and backfat samples, stuffing and samples of Kulenova seka during the process of fermentation were determined. Also, colour and textural parameters and weight loss were determined. For the purpose of monitoring the intensity of fermentation, we conducted continuous measurements of pH value. Fermentation process began immediately after the preparation of the stuffing and lasted for approximately 3 weeks (samples with 0.8% maltodextrin added), approximately 4 weeks (reference sample and samples with 0.8% glucose, sucrose, lactose added) wherein pH value lowers from initial 5.5-5.7 to the lowest value of 5.3-5.4, and 5.0 in the samples of Kulenova seka with maltodextrin added. Instrumental colour parameters and weight losses varied significantly ($p < 0.05$) with the addition of different sugars. Texture parameters were not significantly ($p > 0.05$) affected by the addition of sugars. The sample with addition of maltodextrin showed the highest weight loss during the processing. The results showed that maltodextrin, compared to glucose, sucrose and lactose, had the most effect on the efficiency or speed, intensity and duration of the fermentation process, as evidenced by the most rapid decrease and the lowest pH values, as also with the highest loss in weight loss and the intensity of dehydration (drying).*

***Keywords:*** *Kulenova seka, fermentation, sugars, pH, physico-chemical and sensorial properties*

## Introduction

Fermentation is a method used for meat conservation and it is characterized by several factors: increased number of lactic acid bacteria (LAB) from $10^3$ – $10^5$ CFU/g to $10^6$ – $10^9$ CFU/g, glycolytic sugar degradation and an increase of lactic acid concentration. The increase of lactic acid concentration causes a decrease of pH values from 5.7 at the beginning of fermentation, to 5.5 in slowly-fermented sausages and 4.6 (sometimes even 4.2) in fast-fermented sausages (Varnam and Sutherland, 1995; Toldrá, 2007). Fermentation is most intense during first few hours when the temperature (T) rises to values optimal for LAB growth. This can last from 12 h up to 7 or more days, depending on product type, used additives, production technology and production conditions i.e. temperature and relative air humidity. Higher temperature (T) and higher relative air humidity (Rh) speed up the fermentation process and decrease the pH value. Fermentation can be carried out at temperatures from T = 18 - 24 °C or higher depending on bacteria strains, lasting 1 - 2 days or at lower temperatures (T = 10 - 12 °C) over 1 week. However, in individual cases even at higher temperatures the fermentation process can last over 1 week (Greek and some Italian sausages) (Papamanoli et al., 2003; Comi et al., 2005). Since glucose content in meat is too low or much too variable, and in order to insure the modern industrial sausages production, different carbohydrates like glucose, sucrose, lactose, maltodextrin, corn syrups, starches and sorbitol are added in fermented sausages stuffing. The above mentioned carbohydrates serve as a substrate for the growth of technological micro flora, respectively LAB (Lactic acid bacteria) (Lucke, 1994.). The most common substrate used in this type of fermentation is glucose because glucose is a primary substrate for LAB during their exponential growth phase.

Mass fraction of sugar added and the sugar type, directly affect the speed of decrease and level of pH (Albreht, 2013). Finally, low pH acts as antagonist for pathogens and spoilage bacteria while glucose and NaCl combined increase the osmotic pressure that favors the growth of autochthone technological bacteria. Sugars, mostly glucose, improve the fermentation process in dry sausages because they are used as a substrate for lactic acid production and also contribute to specific aroma development. Maximum 2% (usually 0.3 – 0.8%) of sugars added in fermented sausage stuffing insures the pH value decrease from initial 5.8 - 6.0 to 4.8 to 5.4 (Lucke, 2000.).

The aim of this research was to evaluate the influence of different sugars usually added to fermented sausages stuffing on fermentation process, physical-chemical and sensorial properties of Kulenova seka.

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## Material and Methods

### Sample preparation

The technological process of preparation and production of Kulenova seka was conducted in laboratory pilot plant for dry sausage production and use of ripening chamber with programmable and automated control of technological parameters. Kulenova seka stuffing was prepared according to traditional recipe: pork meat (first and second category 91.8 %), pig backfat (5 %), garlic ( 0.2 % ), red hot paprika powder (0.4 %), sweet red paprika powder (0.6 %) and NaCl (2 %). The stuffing for Kulenova seka is traditionally stuffed in the pig last intestine or in collagen casings (50 cm long and 50 mm in diameter). After the stuffing, the raw Kulenova seka were smoked with dry hard wood (hornbeam, beech and its sawdust) every few day (second to third day, 3 - 4 hours) for two weeks. The temperature and relative humidity at this stage were 18 to 20 °C and 70 to 90 %. After the smoking, the Kulenova seka was left in the ripening chamber with the temperature from 14 to 17 °C and the relative humidity 70 to 80%. The whole technological process, with the processing conditions mentioned above lasted 32 days. A total of 15 samples of raw Kulenova seka were divided into five groups (Table 1).

**Table 1** Samples of Kulenova seka prepared according to traditional recipes with addition of various sugars

| SAMPLE | NUMBER OF SAMPLES | TYPE AND MASS FRACTION OF ADDED SUGAR |
|---|---|---|
| Sample 1 | 3 | 0.8% glucose |
| Sample 2 | 3 | 0.8% sucrose |
| Sample 3 | 3 | 0.8% lactose |
| Sample 4 | 3 | 0.8% maltodekstrin |
| Sample 5 | 3 | Without sugar - Referent sample |

### Physico-chemical analysis

The samples were cut into small pieces and homogenized in a knife mill Gridomix GM 200 (Retsch, Germany). The FoodScan Meat Analyser (Foos, Sweden) was used to determine moisture, total protein, total fat and collagen according to the AOAC 2007.04 method. The pH level was measured in a homogenate of the sample mixed with distilled water (1:10) with pH/Ion 510 – Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/ Oakton Instruments, USA) according to ISO 2917:1999 (HRN ISO 2917:2000) and manufacturer instructions (pH/Ion 510 Instruction Manual). Salt (sodium chloride, NaCl) was determined according to the ISO method 1841:1970. Water activity ($a_w$) was determined using a Rotronic Hygrolab 3 (Rotronic AG, Switzerland) according to manufacturer instructions, at room temperature (20 ± 2 °C). All measurements were conducted in three parallel.

### Texture profile analysis (TPA)

Texture profile analysis (TPA) tests were performed using a Universal TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, GB) equipped with a cylindrical probe P/75. This involved cutting samples in 1.5 cm thick slices, which were compressed twice to 60 % of their thickness. TPA Measurements were performed at a room temperature. Force-time curves were recorded at across-head speed of 5 $mms^{-1}$ and the recording speed was also 5 $mms^{-1}$. The following parameters were quantified (Bourne, 1978): hardness (kg), the maximum force required to compress the sample, springiness (ratio), the ability of the sample to recover its original form after the deforming force was removed, cohesiveness (ratio) the extent to which the sample could be deformed prior to rupture and chewiness (kg) work required to masticate the sample before swallowing, which is calculated hardness · cohesiveness · springiness. Seven measurements were taken from each sample.

### Determination of colour

Colour measurements (CIEL*a*b*) were taken using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The instrument was standardized each time with a and black standard (light trap) and standard white ceramic plate (L*0 = 93.01, a*0 = -1.11, and b*0 = 1.30). The Hunter L*, a*, and b* values correspond to lightness (0 (black) - 100 (white)), greenness (-a*) or redness (+a*), and blueness (-b*) or yellowness (+b*), respectively. The colour measurements were performed on Kulenova seka at room temperature (20 ± 2 °C). Ten measurements were taken from each sample.

### Data analysis

Results are shown as mean ± standard deviation. Experimental data were analyzed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with significance defined at the level of probability of 95 % ($p < 0.05$). Statistical analysis was carried out with Statistica ver. 7.0 StatSoft Inc. Tulsa, OK. USA.

## Results and Discussion

Physico-chemical analysis of meat and back fat (Table 2) used for the Kulenova seka stuffing shows that the mass fraction of basic building blocks (fat, moisture, proteins and collagen) are in accordance with literature data for fresh pork meat and back fat, respectively, 24 h *post mortem*. The water activity and pH values are also in consistence with data of the previous research (Kovačević, 2001). Meat used for dry sausages, but also for other meat products demands pH values 24 h post mortem to be < 6. This indicates the normal course of *post mortal* glycolysis, lactic acid production and pH value decrease.

**Table 2** Basic chemical composition, $a_w$ i pH of back fat, meat and stuffing for Kulenova seka

| Sample | % Fat | % Moisture | % Protein | % Collagen | $a_w$ | pH |
|---|---|---|---|---|---|---|
| Backfat | $74,04^{a} \pm 0,06$ | $19,93^{c} \pm 0,03$ | $7,32^{c} \pm 0,40$ | $2,70^{a} \pm 0,13$ | $0,96^{a} \pm 0,01$ | $6,63^{a} \pm 0,01$ |
| Meat | $9,00^{c} \pm 0,03$ | $70,67^{a} \pm 0,08$ | $19,85^{a} \pm 0,42$ | $1,36^{b} \pm 0,49$ | $0,95^{a} \pm 0,01$ | $5,62^{b} \pm 0,01$ |
| Raw stuffing | $11,86^{b} \pm 0,04$ | $64,69^{b} \pm 0,14$ | $18,84^{b} \pm 0,07$ | $1,11^{b} \pm 0,03$ | $0,94^{a} \pm 0,01$ | $5,51^{c} \pm 0,01$ |

Values are means ±SD of triplicate. Values in the same row with different letters (a-c) are significantly different ($p < 0.05$)

Changes in the mass fraction of individual building blocks and water activity values decrease (from 0.94 for raw stuffing to average 0.86) after 32 days of Kulenova seka production (Table 3), are mostly caused by drying process. During the drying phase the water content decreases from the starting 64.69 % to the average 36 % in all five sample groups. Statistically significant ($p < 0.05$) water content decrease occurred in the sample with 0.8 % maltodextrin.

According to the actual Croatian legislation the maximal moisture content in dry sausage is 40 % (N.N. 131/2012) all samples of Kulenova seka had moisture content below the prescribed level and the values characteristic for this group of fermented sausages (Perez-Alvarez et al., 1999). Mass fraction of NaCl increased, from starting 2 % in raw stuffing, to average value of 3.4 % after 32 days of processing, which is in accordance with investigation results by Ockerman and Basu (2007).

Fermentation and ripening of dry sausages are usually monitored with the changes in pH values (Hagen, et al., 2000; Salgado et al.,2005; Revilla et al.,2005). The changes in pH values during 32 days of processing of Kulenova seka with addition of various sugars are presented in the Figure 1. Results show that after seven days in samples of Kulenova seka with added glucose, sucrose, lactose and in the sample without added sugar (reference) comes to intense decreasing of pH value from the initial pH = 5.55, and after 3-4 weeks reaching the lowest of pH = 5.35 to 5.45 as characteristic value for this type of product (Kovačević, et al., 2011; Kovačević, et al., 2010; Kovačević, et al.,2009). pH values of the sample group with added 0.8 % maltodextrin shows a deviation from the average values of other samples. This is characterized by a drastic drop of the pH value to 5.1 after 14 days, and after 3 weeks of fermentation the pH value decrease to lowest value around 5.0.

Decrease of the pH value is a consequence of the occurrence of lactic acid due to the activities of technological microflora: lactic acid bacteria (LAB) and coagulase-negative staphylococci and the process of glycolysis, i.e. the translation of sugar into lactic acid (Toldrá, 2007). After this the pH values of all samples showed mild increase (Figure 1) which marks the end of fermentation and beginning of proteolytic and lipolytic processes, also known as ripening phase.

Results obtained in this research show significant deviation from literature values, which can be related with specific technological micro flora and specific processing parameters in Kulenova seka processing.

Weight losses in the samples of Kulenova seka during processing are shown in the Table 4. The highest and statistically significant ($p < 0.05$) weight loss occurred in the sample with added maltodextrin. This is a consequence of the most intense pH drop in this sample during fermentation (Figure 1). It can be assumed that the higher drying efficiency in this sample caused higher pH drop which moved meat proteins closer to its isoelectric point and resulted in reduction of meat protein water holding capacity.

CIEL*a*b* system values of Kulenova seka are shown in the Table 5. Addition of various sugars caused statistically significant ($p < 0.05$) differences among samples. Measured colour parameters of Kulenova seka after 32 days were similar to those of Slavonian homemade sausage and Slavonian kulen (Kovačević, et al., 2011; Kovačević, et al., 2010; Kovačević, et al., 2009).

**Table 3** Basic chemical composition, salt content (NaCl), $a_w$ and pH of the Kulenova seka samples after 32 days of processing

| Sample | % Fat | % Moisture | % Protein | % Collagen | $a_w$ | % Salt |
|---|---|---|---|---|---|---|
| Sample 1 | $20,65^{a} \pm 0,08$ | $36,36^{a} \pm 0,37$ | $33,36^{ab} \pm 0,22$ | $3,10^{b} \pm 0,03$ | $0,87^{a} \pm 0,01$ | $3,32^{a} \pm 0,09$ |
| Sample 2 | $20,71^{a} \pm 0,20$ | $36,56^{ab} \pm 0,23$ | $33,20^{b} \pm 0,28$ | $3,09^{b} \pm 0,07$ | $0,87^{a} \pm 0,01$ | $3,39^{a} \pm 0,08$ |
| Sample 3 | $20,37^{a} \pm 0,08$ | $36,68^{a} \pm 0,07$ | $33,79^{ab} \pm 0,19$ | $3,48^{b} \pm 0,11$ | $0,87^{a} \pm 0,01$ | $3,38^{a} \pm 0,09$ |
| Sample 4 | $19,20^{b} \pm 0,37$ | $36,14^{bc} \pm 0,06$ | $34,43^{ab} \pm 0,10$ | $3,35^{b} \pm 0,05$ | $0,86^{a} \pm 0,01$ | $3,35^{a} \pm 0,06$ |
| Sample 5 | $20,74^{a} \pm 0,39$ | $35,76^{c} \pm 0,03$ | $36,57^{a} \pm 0,23$ | $4,19^{a} \pm 0,29$ | $0,86^{a} \pm 0,01$ | $3,58^{a} \pm 0,08$ |

Values are means ±SD of triplicate. Values in the same row with different letters (a-c) are significantly different ($p < 0.05$).

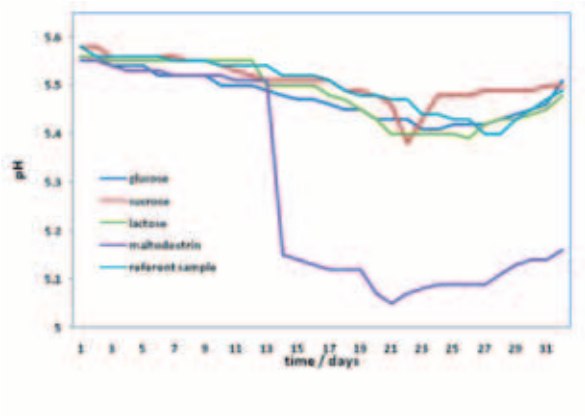


**Figure 1** Changes in pH values during 32 days of processing of Kulenova seka with addition of various sugars (*w* = 0.8 %) and no added sugar (referent sample)

Texture profile analysis (TPA) parameters of Kulenova seka after 32 of processing are shown in Table 6. The hardness, springiness, cohesiveness and chewiness were not significantly ($p > 0.05$) affected by the addition of various sugars.

## Conclusion

The fermentation process of Kulenova seka starts immediately after the stuffing and lasts around 3 weeks for sample with addition of matlodextrin (*w* = 0.8 %) and around 4 weeks for the reference sample and samples with addition of glucose, sucrose and lactose (*w* = 0.8 %). During this period the pH values deceased form round 5.5 - 5.7 to 5.3 - 5.4 and 5.0 for the sample with maltodextrin, respectively. Also, the sample with matlodexrin showed the highest weight loss during 32 days of the processing. Instrumental colour parameters and calculated weight losses varied significantly ($p < 0.05$) with the

**Table 4** Weight loss of Kulenova seka samples after 10, 14 and 32 days of processing

| Sample | Average mass (g) od Kulenova seka sampels at processing days | | | | Weight loss (%) |
|---|---|---|---|---|---|
| | 0 days | 10 days | 14 days | 32 days | |
| Sample 1 | 501,1$^{c}$± 1,94 | 352,7$^{c}$ ± 2,46 | 284,5$^{c}$± 2,01 | 252,0$^{a}$± 2,82 | 49,7$^{d}$ ± 0,42 |
| Sample 2 | 459,3$^{d}$ ± 3,04 | 314,8$^{d}$ ± 2,69 | 251,7$^{d}$± 2,61 | 222,0$^{b}$± 2,23 | 51,7$^{c}$ ± 0,53 |
| Sample 3 | 534,3$^{ab}$± 3,06 | 379,7$^{a}$ ± 5,44 | 293,3$^{b}$± 1,20 | 257,0$^{a}$ ± 4,24 | 51,9$^{bc}$ ± 0,55 |
| Sample 4 | 532,7$^{a}$± 1,62 | 372,7$^{a}$± 1,68 | 310,5$^{a}$± 2,47 | 227,0$^{b}$± 1,41 | 57,4$^{a}$ ± 0,61 |
| Sample 5 | 525,6$^{b}$ ± 0,99 | 363,2$^{b}$± 2,69 | 278,8$^{c}$± 0,85 | 248,0$^{a}$± 1,45 | 52,8$^{b}$ ± 0,39 |

Values are means ±SD of triplicate. Values in the same row with different letters (a-d) are significantly different ($p < 0.05$).

**Table 5** Instrumental colour measurement of Kulenova seka samples after 32 days of processing

| Sample | L* | a* | b* |
|---|---|---|---|
| Sample 1 | 44,00$^{c}$ ± 0,07 | 12,60$^{c}$ ± 0,14 | 26,64$^{c}$ ± 0,08 |
| Sample 2 | 43,55$^{c}$ ± 0,24 | 14,28$^{a}$ ± 0,16 | 25,43$^{e}$ ± 0,15 |
| Sample 3 | 46,97$^{a}$ ± 0,03 | 13,51$^{b}$ ± 0,11 | 27,88$^{a}$ ± 0,14 |
| Sample 4 | 43,16$^{d}$ ± 0,10 | 14,00$^{a}$ ± 0,08 | 26,17$^{d}$ ± 0,08 |
| Sample 5 | 46,19$^{b}$ ± 0,10 | 13,48$^{b}$ ± 0,07 | 27,44$^{e}$ ± 0,06 |

Values are means ±SD of ten measurements. Values in the same row with different letters (a-e) are significantly different ($p < 0.05$).

**Table 6** Textural properties of Kulenova seka samples after 32 days of processing

| Sample | Hardness (kg) | Springiness (ratio) | Cohesiveness (ratio) | Chewiness (kg) |
|---|---|---|---|---|
| Sample 1 | 35,44$^{a}$ ± 2,13 | 0,68$^{a}$ ± 0,08 | 0,57$^{ab}$ ± 0,02 | 13,74$^{a}$ ± 2,08 |
| Sample 2 | 30,28$^{b}$ ± 3,20 | 0,66 $^{a}$± 0,08 | 0,58$^{ab}$ ± 0,04 | 11,59$^{a}$ ±1,89 |
| Sample 3 | 32,19$^{ab}$ ± 4,11 | 0,66$^{a}$ ± 0,11 | 0,59$^{ab}$ ± 0,04 | 12,53$^{a}$ ± 3,34 |
| Sample 4 | 33,90$^{ab}$ ± 4,721 | 0,66$^{a}$ ± 0,11 | 0,55$^{b}$ ± 0,06 | 12,31$^{a}$ ± 3,65 |
| Sample 5 | 33,54$^{ab}$ ± 3,38 | 0,64$^{a}$ ± 0,15 | 0,60$^{a}$ ± 0,05 | 12,88$^{a}$ ± 3,28 |

Values are means ±SD of seven measurements. Values in the same row with different letters (a, b) are significantly different ($p < 0.05$).

addition of different sugars. Texture profile analysis parameters were not significantly ($p > 0.05$) affected by the addition of sugars. The sample with addition of maltodextrin showed highest weight loss during processing. The results of this study showed that addition of maltodextrin ($w = 0.8$ %) to the samples of Kulenova seka, unlike glucose, sucrose and lactose added to the samples in equal ratios, significantly effects the efficiency and speed and intensity of fermentation and lowering of pH value which caused higher weight loss, lower ph value and intensified drying process.

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## OBILJEŽAVANJE SVJETSKOG DANA HRANE 2014.
## Međunarodni skup:
## Kako smanjiti gubitke i bacanje hrane: od polja do stola
## Osijek, 15. listopada 2014.

Hrvatska agencija za hranu (HAH) i Organizacija za hranu i poljoprivredu Ujedinjenih naroda (FAO), pod pokroviteljstvom Ministarstva poljoprivrede, Ministarstva zaštite okoliša i prirode te visokim pokroviteljstvom predsjednika Republike Hrvatske, dr. Ive Josipovića, i ove godine organiziraju nacionalno obilježavanje Svjetskog dana hrane.

Tom će se prigodom, 15. listopada 2014. godine, na Poljoprivrednom fakultetu u Osijeku održati međunarodni skup: Kako smanjiti gubitke i bacanje hrane: od polja do stola.

Izuzetna nam je čast što nam je potvrdio dolazak te će skup otvoriti predsjednik Republike Hrvatske, dr. Ivo Josipović.

Cilj nam je prići ovoj izrazito važnoj temi iz svih perspektiva, stoga smo okupili eminentne predavače, iz Hrvatske i Europe, kao što su prof. dr. Mauro Serafini, voditelj Laboratorija za funkcionalnu hranu i prevenciju metaboličkog stresa iz Rima, Olivier Touze, direktor održivog razvoja Groupement de Mousquetaires "Intermarché" iz Francuske i drugi. Želja nam je da zaključci skupa imaju odraz na realno stanje u Hrvatskoj i doprinesu napretku, u smislu smanjenja gubitaka i otpada hrane.