IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Research Article www.ijafls.org Int J Agric For Life Sci 3(2): 311-319(2019)

Improvement of the rheological properties of wheat flour doughs containing various concentrations of grape seed flour by using glucose oxidase

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Abstract

The purpose of this study was to improve the rheological parameters of the wheat flour dough substituted with grape seed flours (GSF) by using glucose oxidase (GO). GSF were obtained by separating, drying and grinding of the seeds from the grape pomace that is the by-product of wine industry. Öküzgözü (red grape variety) seed flour (ÖSF) and Narince (white grape variety) seed flour (NSF) was added at 0, 5, and 10% levels in replacement with bread wheat flour. GO was added at 0, 50, and 100 ppm concentrations. The rheological properties of the dough, substituted with ÖSF, NSF and GO were recorded in the texture analyser device by using Kieffer dough extensibility system and the Dobraszczyk/Roberts (D/R) dough inflation system. The substitution of ÖSF and NSF was showed a significant decreasing effect on the area (KArea) under Kieffer force–distance curve simultaneously with the increase of the substitution level. Addition of 50 ppm GO in to ÖSF doughs was led to a significant increase at both Kieffer maximum extensibility (Kext) and KArea. (from 12.80 mm to 20.92 mm and from 164.57 g.s to 325.18 g.s respectively) while addition of 100 ppm GO was not had a similar improving effect. On the other hand, improving effect of GO on NSF containing doughs were found at the 100 ppm usage levels of GO by measuring with Kieffer dough extensibility system. Extensional rheology of experimental doughs was measured with the D/R dough inflation system. The substitution of ÖSF or NSF had the significant effect ($P<0.01$) of decreasing the tenacity (P, mm), extensibility (L, mm) and baking strength (W) simultaneously with the increase of the substitution level. The advantage of using GO is clearly evident in dough D/R inflation system values. With the addition of GO, P, L and W values of the doughs increased significantly compared to the ones without GO. In particular, both P and W values of 5% ÖSF or NSF doughs gave similar results with the control sample by using 50 ppm GO. On the other hand, it was found that the rheological properties of dough containing 10% NSF and 10% ÖSF could be partially improved in the case of using 100 ppm GO. As a conclusion, by regarding Kieffer and D/R together; ÖSF or NSF at 5% levels can be added in to bread formulations to benefit their functional properties without impairing dough rheological properties much more. Furthermore, rheological properties of their doughs can be improved by using 50 ppm GO.

Key words: *Bread, dough, D/R dough inflation system, glucose oxidase, grape seed, rheology, Kieffer*

Received: 18.07.2019 **Accepted: 26.11.2019** **Published (online): 26.11.2019**

Introduction

In recent years, grapes have become increasingly popular as an important source of antioxidants such as phenolic compounds, polyphenols, anthocyanins, total dietary fiber, total lipid, magnesium and phosphorus and the importance of these compounds is increasing day by day. Thousands of phenolic compounds with different properties, quantities and functions have been identified in different grape tissues (Rockenbach et al, 2011; Gül et al., 2013; Nunes et al., 2016; Chen and Yu, 2017; Gundesli et al., 2018).

Grape pomace, includes the peels (skins), seeds and, in some cases, the stems, is obtained as a by-product of grape juice and wine processing. Grape pomace accounts apraximately 20-30% of the fruit after processing (Beres et al., 2016). Grape seeds, comprise 38% to 52% of the grape pomace (Costa et al., 2019), on a dry mater basis separated from grape pomace are an excellent source for usage as a food additive because of their valuable nutrient components. 40% fibre, 16% essential oils, 11% protein and 7% phenolic compounds, are found in the proximate composition of grape seeds.

Cite this artile as:
Gul, H. and Ödeş, N. 2019. Improvement of the rheological properties of wheat flour doughs containing various concentrations of grape seed flour by using glucose oxidase. *Int. J. Agric. For. Life Sci.*, 3(2): 311-319.

Grape seeds are associated with also antimicrobial effects (Mohammad et al., 2019), cholesterol-lowering activity (Ngamukote et al., 2011, Kandasamy et al., 2016), cardioprotective properties (Nunes et al., 2016), anti-inflammatory (Pallarès et al., 2013), anticarcinogenic, excellent protection activity against oxidative stress and free radical-mediated tissue injury (Bagchia, et al., 2000), valuable source of antioxidant fibers (Costa et al., 2019) and anti-arthritic effects (Ahmad et al., 2013).

During wine production huge amounts of grape pomace occur which cause storage and environmental problems. However as stated above it has a potential rich source of natural antioxidant and other healthy compounds. Therefore, converting of this by product to a value added product for the industrial and economic point of view is very important. Various studies have been done on the use of grape pomace and grape seed flour (GSF) in different food products particularly in bakery products. Acun and Gül (2014) used grape pomace and GSF in cookie formulations. Grape pomace was used by Gül et al. (2018) as an ingredient to enhance the nutritional and functional properties of glutenfree cookies. Gül et al. (2017a) investigated the effects of grape pomace powder on rheological characteristics of wheat flour dough. Our previous study focused on the consumer acceptability of bread produced from by-products of wine industry (Gül et al., 2017b) indicate that; flours of these by-products, can be used in bread formulations with acceptable consumer preference up to a certain supplementation level.

Bread quality and also rheological properties of the bread dough are negatively affected after a certain additional level of grape pomace or GSF thus, they do not contain gluten. In one of the studies (Mironeasa et al., 2012) it is mentioned that GSF above the level 5% has an increasing effect on amylase activity while has a decreasing effect on dough's consistency.
Deteriorative effects of GSF on rheological properties of dough can be improved by using different oxidative enzymes such as glucose oxidase. Glucose oxidase has a positive effect on rheological properties of bread dough by strengthening of gluten network structure (Gül et al., 2009). Liu et al (2018) reported that continuous and highly dense gluten network was occurred when whole wheat dough treated with GO. Oxidation of β-D-glucose to gluconic acid is catalyzed from glucose oxidase (β-D-glucose:oxygen 1-oxidoreductase; EC 1.1.2.3.4). Molecular oxygen is used as an electron acceptor in this reaction and hydrogen peroxide is produced simultaneously (Bankar et al, 2009). Thus leads to formation of disulfide and non-disulfide bonds.

Most prior studies have focused on the effects of grape seeds flours or extracts on the dough and bread quality. However, to the best of our knowledge studies regarding to improve their effects on rheological properties of dough by using glucose oxidase (GO) have not been undertaken. Therefore, the aim of the study was to evaluate the effects of combinations of both GSF and GO at different concentrations on some rheological properties of bread dough.

Materials and Methods

Materials

Berad wheat flour was purchased from Berberoğlu Gıda Sanayii ve Ticaret A.Ş. (Burdur, Turkey). GSF: Drying and grinding proseses of grape pomace was made according Gül et al. (2013). To put it briefly; white (from Narince grape variety) and red (from Öküzgözü grape variety) grape pomace samples were obtained from wine processing factory (Küp wines Denizli, Turkey) as soon as the winemaking process is completed. Grape pomace samples were dried until reached to 7-8% moisture content during 8-9 hours at 55ºC in a convential oven. Grape seeds were separated from grape pomace manually after drying process. Then grape seeds were ground with hammer mill (Tekpa, Ankara, Turkey), sieved to particle size of ≤300 µm, vacuumed packed and stored at -18ºC until further analysis.

Glucose oxidase: Gluzyme Mono 10000BG (GOX, 10000 GODUF/g) was supplied by Novozymes (Novozymes Dış Ticaret Ltd.Şti, İstanbul, Turkey). All chemicals used were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO,USA), or Merck (Darmstadt, Germany).

Experimental design

ÖSF and NSF were added at 0%, 5% and 10% levels (weight/weight) in replacement with bread wheat flour seperately. GO was implemented as 50 and 100 mg/kg in to each wheat flour- GSF or wheat flour NSF mixture. Doughs prepeared with 100% wheat flour whivh contain no ÖSF, NSF and GO was evaluated as control sample. All experiments were carried out in triplicate.

Methods

Chemical and physicochemical properties of bread wheat flour

Moisture (AACC Metod, 44-01.01), ash (AACC Method 08-01.01), protein (AACC Method 46–12.01), wet and dry gluten and gluten index (AACC Method 38-12.02), sedimentation (AACC Method 56-60.01), falling number (Method 56-81.03), and farinogram (AACC Method 54-21.02) values of wheat flour was determined according to American Association of Cereal Chemists Methods (AACC, 2000).

SMS/Kieffer dough and gluten extensibility test

Rheological properties of doughs prepeared with different amounts of ÖSF, NSF and GO were tested by using SMS/Kieffer Dough and gluten extensibility ring of Texture analyser (TA-XT2, Stable Micro Systems, Surrey, UK) according to the methods of Kieffer et al., (1998). Test parameters; pre-test speed: 2.0 mm/s, test speed: 3.3 mm/s, post-test speed:

10.0 mm/s, distance: 75mm, trigger force: auto - 5g, data acquisition rate: 200pps were applied by using 5 kg load cell. Brief summary of this method as follows: 9.73 g flour or flour mixture was weighed and 0.2 salt was added in to it. Accurate amount of water for each mixture was added. The amount of water added and kneading time were adjusted accordingly to farinogragraph test results. After kneading, dough was roled gently in to a ball. And then in to a sausage shape. Dough was placed on the grooved base form, after placing the top form excess dough was removed, which from the sides with a spatula. This form was cut the dough sample in to strips and allowed the dough to relax, whilst preventing moisture loss. Then dough were relaxed for 40 minutes at a constant relative humidity and temperature (at aroun 24 °C). After relaxation time test was performed with Kieffer rig. Maximum force "resistance to extension", mean distance at max. force 'extensibility' and area under the curve were measured.

Dobraszczyk/Roberts (D/R) dough inflation test

Baking peformance of dough is related to its rheological properties. D/R Dough Inflation test are applied to predict baking performance of dough under strain likes baking expansion. A sheet of dough is inflated with air which is generated by a piston of Texture Analyser. Pressure to inflate of the dough sheet and the volume of the inflated dough sheet are measured (Anonymous, 2019).

The rheological properties of control dough and doughs containing different amounts of ÖSF, NSF and GO were measured using a D/R dough inflation system of a texture analyzer (TAXTPlus; Stable Micro System Ltd., Godalming, UK) according to the procedure established by Dobraszczyk (1997). Brief summary of this method as follows: on the basis of the 100 g flour or flour mixture 2% salt, water according to farinograph water absorbtion values were added. Then these mixutures were kneaded at 25±1 ºC for 6 minutes. Dough samples were placed on the the preperation board and rolled out to 8 mm thickness. Doughs were cut in to 55 mm diameter with circular sample cutter. Doughs were pressed for 30 seconds under the pressure by using press base which subsequently compressed each sample to a fixed thickness of 2.67 mm. Then dough samples were allowed to rest until 30 min. After resting D/R dough inflation system was performed with parameters as; pre-test speed: 8.63 cm^3/sn, test speed: 26.70 cm^3/sn, volume: 2.000.000 mm^3, Trigger volume: 30.000 mm^3). Tenacity (P, mm); the maximum pressure required during inflation of the buble, extensibility (L, mm); the length of the curve up to the point of rupture and baking strength (W); the deformation energy necessary to inflate the sample values of the doughs were measured.

Statistical analysis

Statistical analysis of the doughs prepared with different rates of ÖSF and NSF including different quantity of GO or not were evaluated according to Duncan's multiple range test with significance defined at $P < 0.01$. Analysis of variance (ANOVA) was performed by using the software, statistical package for social science (SPSS 16.0). All the analysis was done in triplicate.

Results and Discussions

Chemical and physicochemical properties of bread wheat flour

Proximate composition and some physicochemical properties of bread wheat flour used for prepearing control doughs and grape seed added doughs are presented in Table 1.

Table 1. Some properties of bread wheat flour

Analysis	Bread Wheat Flour
Moisture (%)	13.23 ± 0.07
Ash (%)	0.61 ± 0.01
Protein (%)	10.44 ± 0.13
Wet gluten (%)	30.65 ± 0.03
Dry gluten (%)	10.29 ± 0.26
Gluten index (%)	93.74 ± 1.28
Sedimentation (ml)	32.3 ± 0.58
Falling number (s)	305 ± 7.00
Farinograph parameters	
Water absorbtion capacity (WAC, %)	62.0 ± 0.1
Dough development time (DDT, min)	1.7 ± 0.14
Stability (STB, min)	12.3 ± 0.54
Softening 12. minute (SFT12, BU)	35.0 ± 8.00

Moisture, ash and protein contents in wheat flour were found as 13.23%, 0.61%, 10.44%, respectively. According to Turkish Food Codex Notification No. 2013/39 on wheat flour (Anonymous, 2018) bread wheat flour should not have to moisture more than 14.5%, ash content of it should be between 0.7-0.8% and protein content at least %10.5, sedimentation at least 26 ml, and falling number should be at least 250 seconds. Wet gluten, dry gluten and gluten index and farinogram values of wheat flour indicated that it is a good quality flour and can be used at bread making. When all chemical and technological characteristics of wheat flour were observed it meets nearly the criteria of Turkish wheat flour standards.

SMS/Kieffer dough and gluten extensibility test results

SMS/Kieffer dough and gluten extensibility test results of both ÖSF and NSF containing doughs with or without GO are presented in Table 2.

Table 2. SMS/Kieffer dough and gluten extensibility values of dough prepeared with different amounts of GSF and GO(1)

GSF (%)	GO (ppm)	Rmax (4) (g)	Extensibility (mm)	Area (g.s)
0 (Control)	0	46.10^{d}	14.49ef	250.14^{ef}
	50	53.00^{c}	16.02^{d}	470.98^{ab}
	100	61.98^{b}	16.06^{d}	497.10^{a}
5 % ÖSF[2]	0	45.17^{d}	12.80gh	164.57^{h}
	50	35.72fg	20.92^{a}	325.18^{cd}
	100	34.21^{g}	12.67gh	200.46fgh
10 % ÖSF	0	37.86ef	13.57fg	194.53fgh
	50	38.54ef	17.90^{c}	210.41fgh
	100	38.34ef	12.22^{h}	179.95^{gh}
5 % NSF[3]	0	37.26efg	19.55^{b}	279.03^{de}
	50	48.07^{d}	21.27^{a}	366.18^{c}
	100	65.20^{a}	17.24^{c}	425.95^{b}
10 % NSF	0	39.97^{e}	13.55fg	155.45^{h}
	50	34.27^{g}	14.94de	191.26fgh
	100	46.43^{d}	13.26gh	240.60efg

(1): There is no statistically significant difference between the averages indicated by the same letter in the same column ($p<0.01$).
(2): ÖSF: Öküzgözü seed flour
(3): NSF: Narince seed flour
(4): Rmax: Maximum force resistance to extension

When compared with the control sample (46.10g) which contain no GSF and GO, the addition of 5% ÖSF did not change the Rmax (45.17g) while increasing the addition rate to 10 % caused a significant decrease ($p<0.01$) in this value (37.86g). On the other hand NSF contributed to the further reduction of Rmax values. However, there was no statistically significant difference between the 5% and 10% addition levels of NSF. Rmax like the stability value of the farinograph parameters is the indicators of the flour strength (Sˇporin et al., 2017), represents how much gluten a flour has and how strong it is . The data obtained in the present study showed that the addition of ÖSF in a level of 5% resulted in a same Rmax values as the control, while, on the other hand at the same addition level of NSF caused a decrease of Rmax values. These results suggest that there is a relationship between the grape variety and rheological properties of the dough. The data obtained are in agreement with the results of Sˇporin et al. (2017), where they reported that the grape cultivar had significant impact on the rheological characteristics of the dough. Rheological properties of dough which supplemented with fibers may be showed difference depending on the botanical origin of the fibre supplement (Miś et al.,2017).

The doughs with grape seed flour from red grape variety (ÖSF) had a higher resistance to extension (Rmax) than samples with grape seed flour from white grape variety (NSF) when they used at 5% level of addition. The data obtained are contrary to data of Iuga et al. (2019), where grape seed flour from white variety for 5% level of addition had a more strengthening effect on dough than samples with grape seed flour from red variety.

As can be seen from Table 2; increasing amounts of ÖSF decreased Rmax. Probably, higher fiber contents of grape seed flours were caused to reduce gluten network formation and thus responsible for reduction of Rmax. Our results are in agreement with those presented by Aghamirzaei et al., (2015) who found that a reduction in stability, and farinograph quality number by increasing the concentration of grape seed flour in the flours.

Although dietary fiber additives have a beneficial effects on human health, their addition in to bread dough cauases a significant reduction at rheological properties of bread dough, which is connected to deteriorative effects of dietary fiber in the structure of gluten proteins (Nawrocka et al., 2016a). Several studies have suggested that the destructive effect of dietary fiber on the gluten network structure (Gül et al., 2009; Wojciechowicz and Gil, 2009; Nawrocka et al., 2016, Han et al., 2019).

The addition of 5% ÖSF reduced the extensibility values of the doughs, whereas the 5% NSF caused a significant rice on extensibility. This results also showed that as in Rmax values, there is a relationship between grape variety (red or white grape) and extensibility values of dough. However, the difference between these two grape seeds was only significant up to the 5% addition level, and when the higher addition rates were reached, the difference between them was insignificant. Another reason why red and white grape varieties have different effects on extensibility may be due to the differences on the solubility of the fibers they contain. More compact gluten network characterized by low extensibility values also obtined in some studies after addition of dietary fibers rich in water insoluble polysaccharides (Miś et al.,2017; Nawrocka et al., 2016a; Nawrocka et al., 2016b; Nawrocka et al., 2016c).

There was no significant difference was found between the extensibility values of control and 10% levels of ÖSF and NSF. When all samples were compared in terms of area values, it was determined that the highest area value was obtained with the use of 5% NSF. This may be arisen from the high extensibility value of this dough sample.

Effects of GO at 0, 50 and 100 ppm usage quantities can be seen from the Table 2. Rmax of the control dough was increased significantly from 46.10 g to 53.0 g and 61.98 g with the increasing rates of GO from 0 to 50 and 100 ppm respectively. This result is in agreement with Steffolani et al (2010), who found that GO increased stability and maximum resistance to deformation (Rm) when enzyme dose increased. GO addition resulted in an increase in dough strength due to its crosslinking effects on gluten proteins.

The extensibility of control dough was increased slightly with the addition of GO. Niu et al. (2018) also reported that the GO enhanced dough strength by increasing the development time, stability, and resistance of whole-wheat dough.

The addition of GO to ÖSF containing flours did not cause a significant change in the Rmax futhermore a limited drop was observed in the samples with 5% ÖSF. The addition of 50 ppm GO to flour containing 5% and 10% ÖSF did not have a significant effect on the Rmax of the dough. However, the addition of 50 ppm of GO gave a statistically significant increase in the area and extensibility values of the both 5% and 10% ÖSF added doughs. On the contrary this positive effect of 50 ppm GO was not seen when its usage levels was increased to 100 ppm. This may be due to the excessive oxidation that may occur as a result of the high levels of GO. Over oxidation leads to a decrease in the elasticity of the dough. This result are in accordance with those of Bonet et al. (2006), who obtained strengthening effect of GO on wheat dough, but they obtained inverse effects when excessive enzyme levels were added. Overcross-linking of the gluten network at high GO concentrations also reported by Meerts et al. (2017), who suggested that the increased resistance of the gluten network towards extension will result in undesirably small bread volumes.

The use of GO was increased the Rmax values of dough samples containing NSF significantly. The positive effect of GO on Rmax and area values was particularly detected when it was used at 100 ppm level.

As a result, since the addition of ÖSF had less negative effect on the rheological properties of the dough than the addition of NSF, the use of 50 ppm GO in the dough containing 5% ÖSF improved the rheological properties. Since the NSF had a more negative effect on the dough structure, it was possible to eliminate this negative effect to some extent by adding 100 ppm of GO.

Dobraszczyk/Roberts (D/R) dough inflation test results

D/R dough inflation values (p, L and W) of dough prepeared with different amounts of GSF and GO are given in Table 3.

Table 3. Dobraszczyk/Roberts (D/R) dough inflation values of dough prepeared with different amounts of GSF and GO(1)

GSF (%)	GO (ppm)	P4 (mm)	L5 (mm)	W6 (Joules*10000)
0 (Control)	0	238.4 cd	52.0 a	512.2 a
	50	402.60def	19.21bc	291.97cde
	100	602.60a	8.19e	270.82de
5 % ÖSF2	0	217.9 cd	13.4 cd	131.9 def
	50	445.43cde	21.38b	421.58ab
	100	514.83abc	10.55de	361.90bcd
10 % ÖSF	0	174.7 ef	7.7 de	78.8defg
	50	347.43f	6.13e	125.24fg
	100	478.20bcd	8.75e	234.29ef
5 % NSF3	0	235.4cd	13.3cd	150.4de
	50	514.58abc	15.53cd	402.99abc
	100	443.90cde	14.72cd	402.72abc
10 % NSF	0	79.2g	9.0de	40.1fg
	50	438.56cdef	5.42e	139.74fg
	100	556.48ab	8.68e	257.35de

(1): There is no statistically significant difference between the averages indicated by the same letter in the same column (p<0.01). (2) :ÖSF:Öküzgözü seed flour, (3) :NSF:Narince seed flour, (4) : P: maximum pressure, (5):L: extensibility, (6) : W: Deformation energy

The addition in increasing rates of GO to the control dough prepared with bread wheat flour was provide a significant increase in the P values whereas it was caused a significant decrease in the L and W values. Similar results were observed by Steffolani et al (2010) who reported that the highest GO level increased maximum resistance to deformation, and decreased dough extensibility. Bonet et al. (2006) observed that the higher levels of GO induced the formation of a discontinuous gluten network.

The addition of GSF to the bread wheat flour was began to reduce the P values significantly after 5% addition levels. There is no difference was determined between the control and % 5 NSF and 5% ÖSF containing doug samples in terms of P values whereas there was a significant reduced was observed at their L and W values. When the usage level of ÖSF and NSF further increased to 10% considerable deterioration on the dough structure was identified. Advantage of GO supplementation in order to partially improve the dough rheological properties which is the purpose of the present study, has been clearly revealed in dough inflation values.

As can be seen in Table 3, addition of GO significantly increased both the P and W values of the GSF containing doughs compared to the ones without GO. The strengthening effect of GO on the GSF added wheat dough has been attributed to the decrease at the number of free sulfhydryls and increase the content of glutenin macropolymer, indicating the formation of additional protein crosslinks via disulfide and maybe phenolic linkages (Bonet et al., 2006; Liu et al., 2018). W values of 5% NSF and ÖSF doughs were yielded similar results with control sample with 50 ppm GO. On the other hand, more positive results were obtained in the case of using 100 ppm GO in 10% NSF and 10 % ÖSF added dough samples.

Generally, when the results of dough iflation test were considered collectively, it was seen that GSF caused a decrease in the rheological properties of the dough due to interruption of gluten network, but at 5% additional level, this effect was less and the negative effect increased as the addition rate increased. This negative effect was improved significantly by using 50 ppm GO for 5 % ÖSF and 5% NSF added dough samples. Niu et al. (2018) suggest that GO could enhance protein polymerization and gluten development in whole-wheat dough.

It was determined that the negative effects of NSF and ÖSF at the 10% suplementation levels could be reduced when the GO ratio was increased to 100 ppm. Even in this case, W values were not reached neither in the control sample nor in the samples which include 5% GSF and 50 ppm GO.

Conclusion

The results demonstrate the deteriorative effects of ÖSF and NSF by products of red wine and white wine processing industry respectively on the rheological properties of wheat bread dough. This weakening impact of GSF has become more evident at the high levels of GSF (10 %) supplementation. Results of the present study also indicates that the reheological quality of wheat flour doughs prepeared with GSF can be improved to some extent by the contribution of GO in to formulation. Rheological properties of bread dough obtained by adding ÖSF or NSF into bread wheat flour at 5% concentration and using GO at 50 ppm would be close to those of control sample values. On the other hand, for the samples added with 10% GSF, the use of 100 ppm GO positively influenced the rheological properties of dough. These results suggested the potential utility of GSF in the production of bread bakery products. When GSF is used up to 5% level a limited weakening effect on rheological quality of bread doughs can be obtained. Furthermore their rheological quality can be improved by adding GO in to bread wheat flour formulations with GSF.

Acknowledgements

We wish to thank to Süleyman Demirel University Scientific Research Projects Coordination Unit (SDÜ-BAP) for their financial support of this study with project number: 3064-YL-12. This paper was presented at the 2. International Conference on "Agriculture, Forestry & Life Sciences" (ICAFLS 2019), April, 18-20, 2019, Prague, Czech Republic.

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