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EFFECT OF CRICKET POWDER ADDITION ON ^{1}H NMR MOBILITY AND TEXTURE OF PORK PÂTÉ

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ABSTRACT

Cricket powder (CP), rich in protein, fat, vitamins and minerals, is potentially a valuable additive for the production of food. While being relatively poorly studied in terms of its technological properties, this raw material has already found acceptance from organizations such as EU or FAO. The research reported in this paper was aimed at investigating the effects of an addition of 2% and 6% of CP to pâté. Proximate composition and textural properties of the products were determined, and Low-Field NMR was used to analyze the impact of the additive on the dynamics of water. Differences were determined in the obtained products in terms of chemical composition, with fat, protein and ash contents increasing with increased addition of CP. The NMR analyzes revealed that 6% addition of CP resulted in an increased ratio of bound to bulk water (decreased T_1 spin-lattice time) and increased mobility of bulk water (increased T_{22} spin-spin time). The same amount of the additive was found to cause a significant decrease in hardness and spreadability of the pâté. A 2% addition of CP did not impact these properties of the product significantly.

Keywords: novel food, texture, low field NMR, meat products, water activity, water dynamics

INTRODUCTION

Pâtés are made of cooked meat, offal, fat and flavor additives. Homogeneous, creamy texture make them a universal product in terms of preparation and consumption. Served hot, they compose well with sauces but can also be used as an addition to sandwiches. Meat, from which pâté is made, is undoubtedly the most important source of protein in diet. It provides the most of the necessary macro- and micronutrients, including exogenous amino acids, fat, minerals and vitamins (**Biesalski, 2005**). In the technological process of producing pâtés, many producers use protein additives due to their emulsifying, gelling, stabilizing and water-binding properties **(Petracci *et al.*, 2013**). The most common are protein preparations from wheat, soy and whey (**Jiang and Xiong, 2013; Marchetti *et al.*, 2014**). There is also a growing interest in enrichment with ingredients that have a positive effect on the health and functioning of the human body (**Jiang and Xiong, 2016**). Cricket powder is one of such additives.
With the estimates concerning the increase of global population and the prospect of widespread problems with food supply, edible insects are looked upon as one of the means to combat the anticipated shortages of protein sources (**FAO, 2012**). Insects have already been used as food in Africa, Latin America or Australia (**Ghosh *et al.*, 2018; Ramos-Elorduy, 1997**), and, as reported by the Food and Agriculture Organization of the United Nations, there are more than 1900 species of insects consumed worldwide. Crickets, mealy larvae, ants, grasshoppers and flies are some of the examples (**van Huis, 2013**). Some European countries have regulated the production of insect-based foods in the past, but from 1st January 2018, insects as well as their parts can officially be regarded as the so-called "novel food". The change in legislation in this area may contribute to the increase of insect consumption in Europe (**C/2017/8878**).
Because of their significant content of protein, vitamins and minerals, crickets show high nutritional value and may be considered a valuable additive in the production of food **(Ayieko *et al.*, 2016; Duda *et al.*, 2019; Montowska *et al.*, 2019; Pauter *et al.*, 2018; Stull *et al.*, 2018**). Therefore, the aim of this work was to evaluate the influence of cricket powder on the texture and water mobility of pâtés supplemented with it.

MATERIAL AND METHODS

Pâté manufacturing

Pâtés were prepared using meat, liver and fat from pork (Mas-Pol, Warsaw, Poland) as well as commercially available cricket powder (Crunchy Critters, London, United Kingdom) and a mix of spices consisting of pepper, dried onion and marjoram (McCormick Polska S.A., Stefanowo, Poland). Pork meat and fat were boiled in water until tender. The material prepared in this way was pre-grated in a PT-98 type mincer (Mainca, Barcelona, Spain) using a 3 mm mesh. After that, the meat was combined with the hot broth obtained during cooking of meat and spice mix, and mixed in a bowl cutter (CR-22, Mainca, Barcelona, Spain) at 55 °C. At this stage, cricket powder was also added. Homogenized pork liver was added to the minced meat and the grinding operation was continued until homogeneous consistency was obtained. Finally, the mass was placed in 200 mL jars and cooked at 70 °C for 40 minutes. Thus prepared pâtés were cooled with cold water and stored in a refrigerator prior to the analyses. Based on previous consumer attractiveness studies (**Smarzyński *et al.*, 2019**), 2 levels of CP addition were established. The pate formulations contained 0%, 2% and 6% addition of cricket powder, and were termed R, CPP2 and CPP6, respectively. Detailed composition of the three tested product variants is presented in Table 1.

Table 1 Recipes of the tested and reference pâtés

Ingredient	R [%]	CPP2 [%]	CPP6 [%]
Pork meat	38.96	38.18	36.62
Broth	19.48	19.09	18.31
Pork fat	24.35	23.86	22.89
Pork liver	14.61	14.32	13.74
Spice mix	2.6	2.55	2.44
Cricket powder	0	2	6

Legend: R, CPP2 and CPP6 denote pates with cricket powder at 0%, 2% and 6% (w/w), respectively.

Chemical composition of pâtés

Total nitrogen content was determined by the Kjeldahl method according to PN-EN ISO 8968 and was used to calculate the protein content by multiplying the result by the conversion factor of 6.25. The fat content (Soxhlet method) was determined according to PN-ISO 1444, total water content – according to PN-ISO 1442, ash content – according to PN-ISO 936, pH – according to ISO 2917. Measurements of water activity were performed according to the method described by Baranowska et al. (2017) using a LabMaster-aw neo analyzer (Novasina AG, Switzerland). Before the measurement, the temperature was stabilized to 20.0 °C with an accuracy of ± 0.1 °C.

NMR relaxometry

NMR measurements were performed according to **Baranowska *et al.* (2018)**. The volume of pâté samples was 1.6 cm^3. The inversion-recovery ($\pi-t-\pi/2$) (**Brosio and Gianferri, 2009**) pulse sequence was applied for measurements of the T_1 relaxation times. Calculations of the spin-lattice relaxation time values were performed with the assistance of the CracSpin program.
Measurements of the spin-spin (T_2) relaxation times were taken using the pulse train of the Carr-Purcell-Meiboom-Gill spin echoes ($\pi/2-\tau/2-(\pi)_n$) (**Brosio and Gianferri, 2009**).
In all the analyzed systems the one spin-lattice T_1 and two spin-spin T_2 components were obtained. These results were used for characterization of meat products **(Baranowska, 2011; Bertram *et al.*, 2003; Shao *et al.*, 2016)**.

Texture analysis

Texture was analyzed using a TA.XTplus texture analyzer (Stable Micro System Co. Ltd., Surrey, England) equipped with a 5 kg load cell and the TTC Spreadability Rig made of plexiglass (HDP/SR, Stable Micro System Co. Ltd., Surrey, England). The angle between the base of the cone and its generatrix was 45º. A data acquisition rate of 200 measurements per second was used. The probe was moving at 3.0 mm/s. The measurements were carried out at 22 ± 2 ºC.
The analysis consisted of two phases. During the first phase of the test, the sample, placed in a static cone-shaped container, was penetrated with the upper cone to a depth of 23 mm. The maximum value of force recorded by the device during this test phase is referred to as hardness [N] while the area under the obtained curve of force vs time is termed spreadability [N·s]. During the second test phase, the head moved in the opposite direction. The maximum force recorded during this phase of the test is referred to as stickiness [N], while the area under is termed adhesion [N·s].

Statistical analysis

All the measurements were repeated three times, unless stated otherwise. One-way analysis of variance (ANOVA) was carried out independently for each dependent variable. Post-hoc Tukey HSD multiple comparison test was used to identify statistically homogeneous subsets α = 0.05. Statistical analysis was performed with Statistica 13 software (Dell Software Inc., USA).

RESULTS AND DISCUSSION

Cricket powder (CP) is a high-protein product that also contains significant amounts of fat (**Ayieko *et al.*, 2016; Montowska *et al.*, 2019**). Therefore, its inclusion in the recipe of pâté resulted in a significant increase in protein content in the finished product. As could have been expected, the increase was the higher the greater the added amount was (Table 2). A similar trend was noted in the case of fat content. Crickets were found to provide protein, minerals and vitamins as well **(Montowska *et al.*, 2019; Stull *et al.*, 2018; Zielińska *et al.*, 2015**). It was also noted that the pH value of pâté with the highest additive level (CPP6) was lower than in the case of the other samples. Addition of CP caused an increase in the content of total ash in pâtés as well but did not affect the total water content. No effect of CP additive on the value of water activity was found. Alteration of the chemical composition resulted in changes in the water mobility and texture of pâtés.

Table 2 Basic characteristics of pâtés

Parameter	R	CPP2	CPP6
Protein content [%]	15.97 ± 0.25^c	17.45 ± 0.23^b	19.22 ± 0.09^a
Fat content [%]	27.46 ± 1.02^b	28.29 ± 0.99^{ab}	30.68 ± 1.28^a
Ash content [%]	1.80 ± 0.03^c	1.95 ± 0.04^b	2.02 ± 0.06^a
Total water content [%]	43.64 ± 3.02^a	44.96 ± 3.40^a	43.54 ± 2.30^a
pH	6.36 ± 0.02^a	6.30 ± 0.05^a	6.20 ± 0.06^b
A_w	0.9690 ± 0.0007^a	0.9659 ± 0.0012^a	0.9667 ± 0.0006^a

Legend: R, CPP2 and CPP6 denote pates with cricket powder at 0%, 2% and 6% (w/w), respectively.
Mean values in rows and denoted by different letters differ statistically significantly ($p < 0.05$).

The results obtained with the Low Field NMR method allow to determine the molecular dynamics of water of an analyzed system **(Li *et al.*, 2014; Marcone *et al.*, 2013**). One component of the spin-lattice relaxation time and two or three components of the spin-spin relaxation time are usually observed in meat products (**Bertram *et al.*, 2003; McDonnell *et al.*, 2013**). In the case of the measurements carried out with use of a spectrometer operating at a relatively high frequency and taking into account low power RF pulses, only bulk and bound fractions of water relaxation are observed in the studied systems of meat products. Thus, only two components of the spin-spin relaxation time were observed. The results of NMR analysis are presented in Table 3.

Table 3 The values of the spin-lattice T_1 and short T_{21} and long T_{22} components of spin-spin relaxation times in pâtés

Parameter	R	CPP2	CPP6
T_1 [ms]	159.9 ± 0.4^a	151.5 ± 0.5^b	130.8 ± 0.4^c
T_{21} [ms]	20.1 ± 1.0^a	18.9 ± 0.8^b	19.1 ± 0.9^{ab}
T_{22} [ms]	71.8 ± 2.0^b	70.2 ± 1.8^b	86.2 ± 3.9^a

Legend: R, CPP2 and CPP6 denote pates with cricket powder at 0%, 2% and 6% (w/w), respectively.
Mean values in rows and denoted by different letters differ statistically significantly ($p < 0.05$).

The spin-lattice relaxation time reflects the mutual relations between the amount of free and bound water in the system. The values of this parameter are strongly dependent on the Larmor frequency. In addition, the spin-lattice relaxation time increases with increasing water content in the system. Spin-spin relaxation time is a parameter that characterizes the dynamics of water molecules in the matrix. The longer the time observed, the greater is the mobility of water protons. In the presence of two fractions of relaxing protons with different spin-spin times, the mobility of bound and bulk fractions can be analyzed separately. Obtained values of relaxation times are typical for the tested material at a frequency of 30 MHz (**Baranowska, 2011; Piątek *et al.*, 2013**).
Addition of 2% of CP did not impact significantly the ratio of bound to bulk water and the molecular dynamics of both these fractions. This was indicated by the relatively small differences of T_1 and T_{21} relaxation times between R and CPP2 samples. It was, however, determined that the addition of 6% of CP had significant impact on both the T_1 and T_{22} relaxation times. These changes were reflected by the results of texture analyzes discussed further. CP addition in the amount of 6% was found to significantly increase the content of protein and fat in the pâté. As protein acts as a water holding agent, the T_1 relaxation time was increased reflecting the increased ratio of bound to bulk water. Moreover, the increased mobility of bulk water molecules, represented by the T_{22} relaxation time, was likely a result of the introduction of a hydrophobic factor (fat) to the system. Small differences in T_{21} among the tested samples indicated similar dynamics of the bound water fraction in all the tested samples.
Pâté is composed of proteins (both soluble and insoluble), droplets of fat, water, salt and spices. When raw, this complex mixture forms a paste. During cooking it forms a more rigid structure as the proteins start to form a gel. The final structure is obtained when proteins denature and protein-protein interactions form (**Barbut *et al.*, 1996**). Fats of animal origin are rich in saturated fatty acids while insect were found to be rich in unsaturated fatty acids (**Zielińska *et al.*, 2015**). The addition of CP to pork pâté can therefore improve the profile of fatty acids found in this product shifting the quantitative balance of fatty acids towards mono- and polyunsaturated acids. This could be beneficial in terms of the general effect of the product on the health of consumers. On the other hand, as fat is responsible for the rheology of batter and the final product (**Steen *et al.*, 2014**), care has to be taken when designing pâté with modified composition. Texture analysis showed

that the hardness of pâté was decreased significantly only in the case of the product with 6% CP addition (Table 4). This was accompanied by a 10% decrease in spreadability when compared to the reference pâté. Application of animal fat substitutes containing unsaturated fatty acids has already been reported to decrease the hardness of pâté (**Martin *et al.*, 2008; Terrasa *et al.*, 2016**). Moreover, myofibrillar proteins are responsible for the emulsifying capacity in meat products. They facilitate the formation of a shell on the surface of fat droplets which has decisive impact on the structure of a product. This structure has a significant impact on its texture and stability (**Adamczak and Szczeblewska, 2004).** The effectiveness of myofibrillar proteins in shaping the texture was also found to be dependent on the production technology (**Micklisch *et al.*, 2005**). While the addition of 2% of CP allowed to obtain a product with properties of the conventional product preserved, the higher addition amount tested resulted in significant changes to most of the analyzed textural parameters. However, in the scope of current knowledge, it seems likely that higher amounts could be introduced without the negative effect on texture if enzymatic treatment of the additive was used as proteolysis was found to improve the emulsifying properties of cricket proteins (**Hall *et al.*, 2017**).

Table 4 Results of texture analysis of pâtés

Parameter	R	CPP2	CPP6
Hardness [N]	34.65 ± 1.93[a]	32.16 ± 2.72[ab]	30.10 ± 1.13[b]
Spreadability [N·s]	62.37 ± 8.31[a]	58.15 ± 7.46[a]	56.29 ± 4.73[b]
Stickiness [N]	-35.38 ± 2.94[a]	-31.15 ± 2.39[a]	-28.21 ± 1.51[b]
Adhesion [N·s]	-4.01 ± 0.32[a]	-2.71 ± 0.34[b]	-2.66 ± 0.54[b]

Legend: R, CPP2 and CPP6 means pates with cricket powder at 0%, 2% and 6% (w/w), respectively.
Mean values in rows and denoted by different letters differ statistically significantly ($p < 0.05$).

CONCLUSION

The presented results indicate that CP can be implemented in the production of pâté and that it is possible to obtain a final product with textural properties similar to those of the conventional product as long as the additive is not used in excess. The 2% addition was found not to influence the texture to a significant extent. In general, CP addition to pâté was found to increase the content of protein, fat and ash in the final product. In case of the 6% addition of CP the dynamics of water were influenced. Based on decreased T_1 spin-lattice relaxation time it can be stated that the ratio of bound to bulk water was increased in this case. Moreover, the mobility of the molecules of bulk water was increased as indicated by the T_{22} spin-spin relaxation time in the samples with 6% CP. These were likely the results of the increased protein content and altered profile of fatty acids. No changes were, however, noticed in the dynamics of the water molecules in the fraction of bound water. The altered composition also resulted in changes to the textural properties of the final product. The samples of pâté that contained 6% CP showed decreased hardness and spreadability values.

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