THE INFLUENCE OF SPICES ON THE FERMENTATION OF A BELGIAN DRY SAUSAGE

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INTRODUCTION

The pH is a generally known central parameter during dry sausage ripening. The necessity of a pH decline to obtain an acceptable texture, color development and shelf life has to be considered as an established fact (Ten Cate, 1960; Klement et al, 1974; Klement et al, 1975). Various acidulants are used in practice to control and/or enhance this pH decline. In this respect the addition of easy fermentable carbohydrates and starter cultures, including members of the Lactobacillus, Pediococcus, Streptococcus and Micrococcus genera, are well known practices. A direct acidulation is achieved with glucono-delta-lacton (GDL) because of its hydrolysis into gluconic acid in the aquenous sausage system.

Spices are traditionally used as seasonings in sausage production. Besides their obvious function as flavour compound other properties such as antioxidative and antimicrobial action have been attributed to spices (Hammer, 1977; Salzer et al, 1977; De Wit et al, 1979). Zaika et al (1978) have recently observed accelerated pH decline due to the addition of some spices to Lebanon-Bologna sausage. The latter however is a heat processed sausage as contrasted with European dry sausage. The aim of this work was to investigate the influence of spices on the ripening of a Belgian dry sausage.

MATERIALS AND METHODS

1. Preparation of sausages - In 3 experiments a total of 15 series of 5 to 10 sausages each was prepared in the laboratory. Each serie was prepared from cooled, deboned and chopped beef (800 g), pork (600 g) and lard (600 g) to which additives (13.2 g glucose, 60 g NaCl, 0.2 g $NaNO_2$ and 1 g Na-ascorbate) were added. Each experiment contained a serie without added spices and as indicated in the tables, series with 0.5% or 1% spices mixture 1 analoguous to the mixture used by Zaika et al (1978) (Mix. 1 containing 2.86 g black pepper, 1.43 g nutmeg, 1.43 g allspice, 0.71 g red pepper, 0.71 g clove, 0.71 g cinnamon, 0.71 g ginger, 0.71 g mustard, 0.71 g majoram and 0.02 g mace), 0.5% spices mixture 2 typical for a Belgian dry sausage (Mix.2 containing 7.6 g black pepper, 1.5 g mustard and 0.9 g mace), various paired combinations of mix. 1 spices as indicated in table 3 using the same respective amounts as in mix. 1, a mixture identical to mix. 1 but using organic extraxts on carrier sucrose (obtained from Coene, Brussels) and an aqueous extract of mix. 1. The latter was prepared treating mix. 1 with 50 ml 6% NaCl in H_2O (w/v) at 40°C for 2 h, filtration and lyophilization. The residue was dissolved in 10 ml of H_2O before use. To sausage series in the same experiment, 10 ml H_2O was added.

Beef, pork, additives and spices were cuttered during 1 min 15 sec (11 rpm)(Rex Laufach cutter, Germany). After addition of the frozen lard, the mixture was cuttered again for 1 min 45 sec. The temperature of the mixture did not exceed -1°C. Naturin casings (diam. 55 mm) used for stuffing, were soaked for 1 h in an aqueous pimaricin solution (1000 ppm). Pimaricin was obtained from Ter Beke N.V., Waarschoot, Belgium.

After stuffing, using a hand operated stuffer, the sausages (250 g) were submitted to the following ripening conditions (Klimaschrank Type 500, Carl Weiss, Giessen, Germany) :

Days after stuffing	Temperature (°C)	Relative humidity
0 - 2	24	95
2 - 3	24	92
3 - 8	20	85

Air velocity was $\leq$0.2 m/sec, relative humidity was measured and regulated using saturated solutions of respectively K_2SO_4, Na_2CO_3, KCl.

Sausages were not smoked and no starter cultures were used.

2. Analysis - After stuffing sausages were sampled daily (expt. 1) or at 0;2;4;6; and 8 days. pH was measured by insertion into a sausage of a pointed combinated glass-reference electrode (Ingold, Zurich) connected to a Knick Portamess 651 digital pH-meter (Knick, Berlin). Average values of 5 readings at different sites were calculated (mean variation coefficient <1%). Dry matter was measured in triplicate on 5 g by drying for 4 h at 105°C. Texture was measured as cohesiveness and hardness (Friedman et al, 1963) on a transverse section of sausage (15 mm thickness) using an Instron Universal Testing Machine (Instron Ltd, High Wycombe, G. Britain).

Acetate was determined after steam-destillation as described in De Katelaere et al,(1974). Extracts in 0.6 $NHClO_4$ were prepared in triplicate (De Keteleare et al, 1974) and used for determination of total carbohydrates expressed as hexose (Herbert et al, 1971), lactic acid and ammonia (Conway, 1957). Bacterial counts (expt. 1 only) were performed as described earlier (De Keteleare et al, 1974) but incubation temperature was 28°C. Significant lower counts were found at 38°C (data not shown).

RESULTS AND DISCUSSION

1. Presentation of data - The experiments involved 15 series of dry sausage ripenings, each of which was sampled 5 to 7 times for determination of dry matter (D.M.), pH, total carbohydrate, lactate and ammonia-N. In two experiments, texture was evaluated by measurement of cohesivness and hardness.

Comprehensive presentation of data is necessary to allow comparison and eventual statistical evaluation of the effect of spices. Therefore each sausage ripening was characterized by regression coefficients obtained by curve fitting of measured values against time. Visual inspection showed that change in total hexose concentration and pH could be fitted with acceptable accuracy by regression against time following an exponential equation $y = b \cdot e^{ax}$ with x = days after stuffing. The coeffecient a is a measure of the fractional rate of change whereas b is an estimate of the initial values. Linear regression against time was used for changes in D.M., lactate and ammonia concentration following y = ax + b, a being a measure of the rate of change, b an estimate of the initial values. With D.M. and ammonia the linear relationship was apparent on visual inspection. Net lactate production could also be modelled following $y = a \cdot x^b$ but variation in initial and final production rates was large, and associated with discrepancies between hexose disappearance and lactate production. Therefore it was judged better to estimate average overall rate of lactate production by linear regression. Mean determination coefficients (R^2) were 0.87, 0.92, 0.87, 0.89 and 0.99 for D.M., total hexose, lactate, pH and ammonia-N respectively. An example of curve fitting is shown in fig. 1. It is clear that the models used do not account for some typical changes in dry sausage, namely :

- -the slight decrease in D.M. during the first days of ripening (De Ketelaere et al, 1974)
- -the slight increase in pH at the later stages of ripening (Demeyer et al, 1979)
- -the possible role of lactate as an intermediate being formed and metabolized (De Ketelaere et al, 1974)

Nevertheless it was accepted that regression coefficients obtained using the models characterize overall rates of changes with sufficient accuracy to be used in the evaluation of the effect of spices. The difference observed between values obtained after 0 and 8 days of ripening were used as estimates for the extents of changes. When sufficient data were available, the effect of spices was evaluated using the paired t statistic on rates and extents of changes.

2. Effect of spices on drying of sausage - Data in table 1 clearly show that the Lebanon spice mixture used (mix. 1) significantly increased both rate and extent of drying. The mechanism of this effect is not clear. The Belgian mixture (mix. 2) appears to have the same effect whereas the data suggest that the active factor is soluble in both water and organic solvents, although too little data are available to allow a definite conclusion. Black pepper and cloves clearly is the most active binary combination used. As cloves are absent from the Belgian mix. 2, the data suggest black pepper as being the most active component. This may be due to the relative large amount of black pepper used and not to any specific difference between the spices.

3. Effect of spices on carbohydrate metabolism, change in pH and ammonia-N production - The data in table 2 show that the Lebanon-Bologna mixture (mix. 1) significantly increases both rate and extent of carbohydrate metabolism and fall of pH. Although the average rate of lactate production is increased, the difference is not statistically significant. Extent of lactate production was significantly increased however. The stimulatory effect of the spice mix. 1 on hexose metabolism and lactate production was not associated with an increase in the numbers of lactobacilli and micrococci present (fig. 2). Also, the effect was not due to microbial contamination of the spices as addition of spices before and after sterilization (30 min 100°C) to nutrient broth agar (Difco 800mg/300ml), containing sausage additives in concentrations similar to the sausages did not result in significant changes in pH, total carbohydrate or lactate concentration (data not shown). Incubation conditions were those of sausage ripening (see materials and methods).

2). Binary combinations of spices showed the combination black pepper + cloves to be most active, followed by allspice + ginger. As cloves were absent from the Belgian mixture the data suggest black pepper as being the most active component (table 3). Again the effect is probably due to the relativley high concentration used, rather than to a specific property of black pepper.

The accelerated fall in pH is obviously related to the increase in lactate production : the extent of pH decline (x) was related to the extent of lactate production (y) following $y = 8.16 x + 1.88$ ($R^2 = 0.72$; $p \leq 0.001$). Also rate of lactate production was significantly correlated to rate of pH fall ($R^2 = 0.64$; $p \leq 0.001$). In earlier work we established that pH in dry sausage is mainly determined by lactate and ammonia concentration (Demeyer et al, 1979). In line with these findings a highly significant linear relation was found between extent of pH fall (y) and log ($[NH_3] / [\text{lactate}] .100$), (x) where $[NH_3]$ and [lactate] stand for the extent of ammonia formation and lactate formation respectively (mmoles/100 g D.M.) : $y = 2.49 - 1.23 x$ with $R^2 = 0.88$ ($n = 12$; $p \leq 0.001$) (fig.3). This in contrast with linear regression between extent of pH fall (y) and log [lactate] (x), with [lactate] = extent of lactate formation in mmoles/100 g D.M. : $y = 1.4 x - 0.5$ with $R^2 = 0.80$ ($n = 15$, $p \leq 0.001$). Extent of lactate production (y) was related to hexose disappearance (x) following $y = 0.58 x + 1.78$ but the relationship was poor ($R^2 = 0.43$, $p \leq 0.01$). Also rate of lactate production was correlated with rate of hexose disappearnace ($R^2 = 0.41$, $p \leq 0.05$). The poor relationship between hexose disappearance and lactate production is obviously associated with the variable and low recovery of hexose as lactate : extent of lactate production only accounts for 0.69 ± 11 (mean value $\pm$ S.E. of 15 values) of hexose disappeared. There was no effect of spices on this low recovery. Occasional determination of acetate indicated amounts between 1 and 2 mmoles per 100 g D.M. These data suggest that considerable amounts of hexose were completely oxidized to CO_2 and H_2O, in line with earlier suggestions (De Ketelaere et al, 1974; Pezacki & Fischer, 1966). Such oxidation may be related to the use of a non-vacuum filling device.

4. Effect of spices on texture of dry sausage - Cohesiveness and hardness were measured in experiments 3 and 4 and found to be increased by the addition of spices (fig. 4). It is reasonable to assume that such increases are related to the accelerated fall of pH and drying rate respectively. Indeed, salt soluble myofibrillar proteins gelating as pH 5.2 will contribute to cohesiveness (Klement & Cassens, 1973) and such pH will be reached earlier in ripening when spices are added (fig. 4). Also using other data obtained later in the ripening period of expt. 3, a significant positive correlation could be calculated between hardness and % D.M. ($R^2 = 0.81$, $p \leq 0.001$). The exact quantitative relationship between values of cohesiveness and hardness and acceptability of sausage texture is not known and will be further investigated. It is clear however that addition of spices does allow an increase of both texture characteristics, earlier in the ripening period, related to an accelerated fermentation and drying.

SUMMARY

The data reported confirm earlier work of Zaika et al (1978), Kissinger & Zaika (1978) and Zaika & Kissinger (1979). Spices do stimulate carbohydrate fermentation to lactate with concomittant acceleration of pH decrease. This effect is not due to microbial contamination of spices and no increase in bacterial numbers in the sausages could be detected. The nature of the stimulating component is unknown but the data suggest it to be water soluble. The findings of Zaika et al (1978) have been extended to low temperature fermented sausage whereas an additional effect, namely accelerated drying, was demonstrated. The importance of these effects for development of texture was demonstrated.

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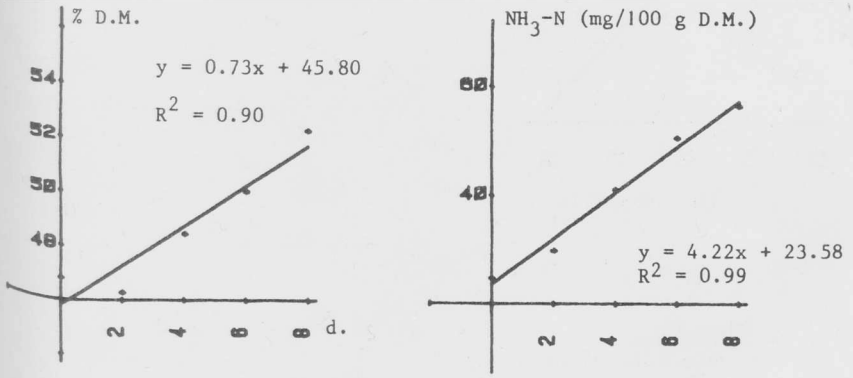
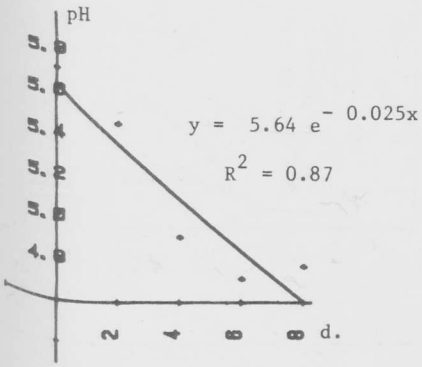
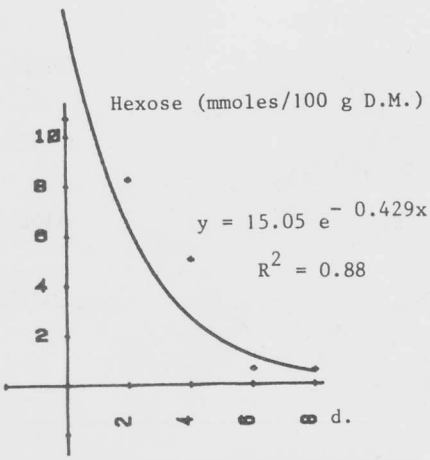


Fig. 1 : Characterization of sausage ripening by linear and exponential curve fitting (data from expt. 2 with 0.5% spice mix. 1)





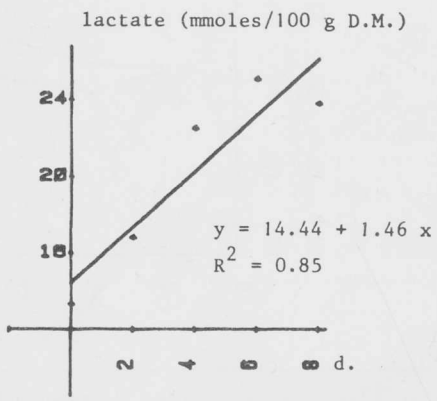


Fig. 2 : Numbers of lactobacilli and micrococci (exp. 1)

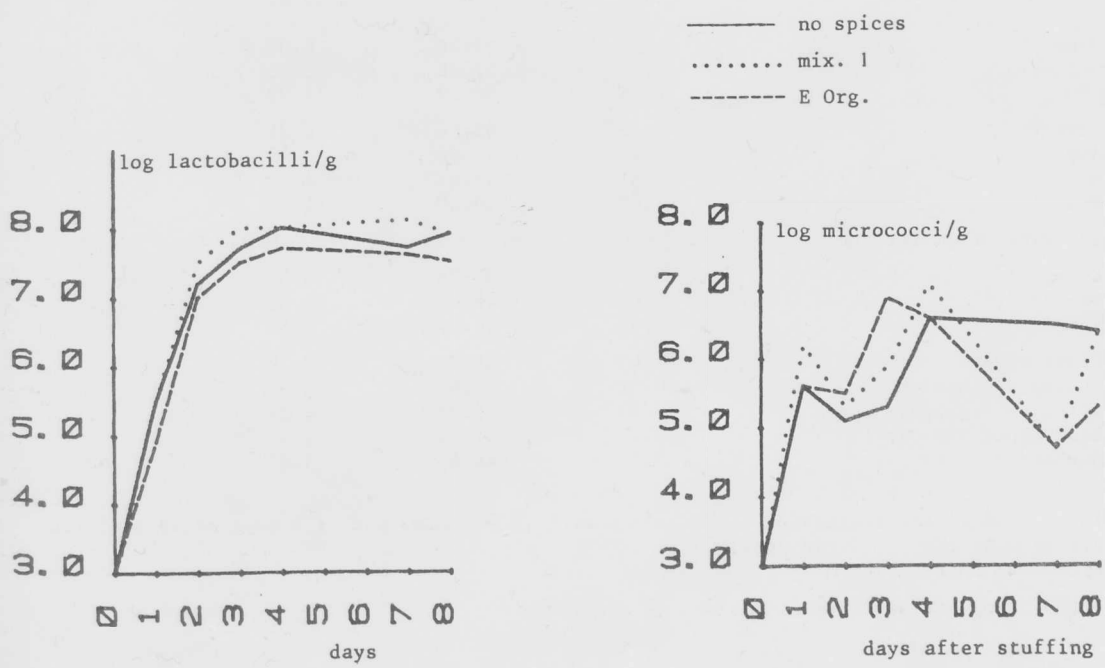


Fig. 3 : Relation between extent of pH change and extent of lactate and ammonia production

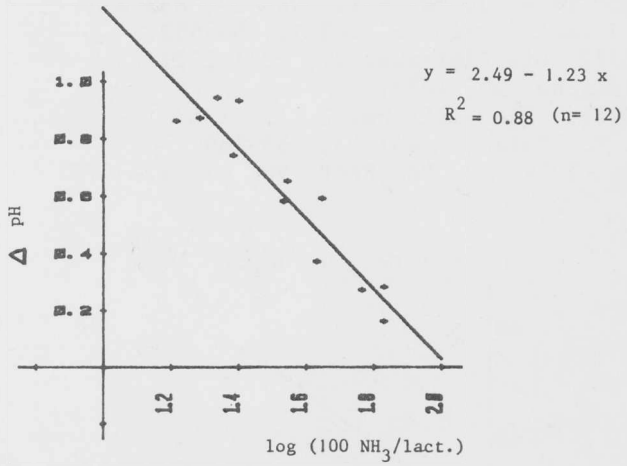


Fig. 4 : Effect of spices on sausage texture

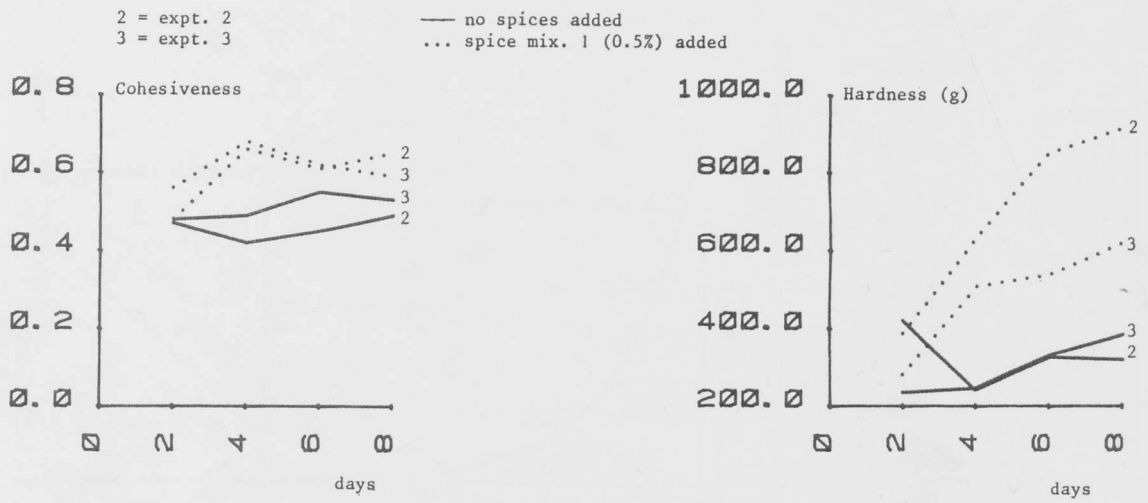


Table 1 : Effect of spices on rate and extent of drying

Expt.	Spices added	Change in % dry matter		
		a[1]	b[1]	total
1	None	0.87	53.20	6.00
2	"	0.46	52.88	3.38
3	"	1.26	50.69	8.99
1	Mix. 1	1.16	52.44	8.99
2	Mix. 1	0.73	45.80	5.43
	Mix. 1[3]	0.58	49.63	4.87
3	Mix. 1	1.48	49.40	11.43
Significance level of difference[2]		$p \leq 0.01$		$p \leq 0.01$
1	E org.	1.15	53.88	8.57
2	Mix. 2	0.60	48.71	4.38
3	E H_2O	1.34	54.07	9.58
2	Black pepper + cloves	0.62	48.79	7.30
	Nutmeg + cinnamon	0.51	50.94	3.99
	Allspice + ginger	0.63	49.21	4.91
	Red pepper + marjoram	0.43	51.35	2.90
	Mustard + mace	0.70	46.65	5.03

[1] Calculated from linear regression $y = ax + b$ with y = % D.M. and x = days after stuffing
a = rate of D.M. change (% D.M./day)
b = calculated initial % D.M.

[2] Calculated using the paired t statistic

[3] 1% of spice mix. 1 added

Table 2 : Effect of spices and spice extracts on rates of carbohydrate removal, lactate and ammonia production and change of pH

Expt.	Spices added	Carbohydrate removal			Lactate production			NH_3-N production			Change in pH		
		a[1]	b[1]	total	a[2]	b[2]	total	a[2]	b[2]	total	a[1]	b[1]	total
1	none	- 0.095	9.65	4.45	0.69	11.68	5.09	-	-	-	- 0.011	5.64	0.44
2	"	- 0.090	8.73	3.97	0.52	9.71	4.21	3.66	6.23	34.2	- 0.006	5.77	0.27
3	"	- 0.131	8.16	5.16	0.67	8.57	5.15	2.94	29.53	24.5	- 0.014	5.77	0.58
1	Mix. 1	- 0.356	12.11	7.75	1.84	11.18	12.96	-	-	-	- 0.025	5.81	1.05
2	Mix. 1	- 0.429	15.05	10.16	1.46	14.44	10.46	4.22	23.58	32.0	- 0.025	5.64	0.94
	Mix. 1[3]	- 0.215	12.49	8.34	1.34	10.39	9.27	4.27	14.56	32.7	- 0.024	5.89	0.93
3	Mix. 1	- 0.385	8.22	8.02	0.73	11.85	6.61	2.62	33.12	17.8	- 0.023	5.51	0.87
Significance level of difference :		$p \leq 0.05$		$p \leq 0.05$	N.S.		$p \leq 0.05$	N.S.		N.S.	$p \leq 0.01$		$p \leq 0.01$
1	E org.	- 0.046	11.24	3.94	1.13	8.58	8.80	-	-	-	- 0.012	5.71	0.52
3	E H_2O	- 0.148	11.07	6.49	0.96	8.77	7.16	2.27	19.10	16.4	- 0.019	5.81	0.86
2	Mix. 2	- 0.303	8.00	6.11	1.12	9.55	8.12	3.73	6.18	27.5	- 0.023	5.58	0.74

[1] Calculated from exponential curve fit $y = b \cdot e^{ax}$ with y = mmoles/100 g D.M. or pH, x = days after stuffing, b = calculated initial concentration or pH and a = fractional rate of change

[2] Calculated from linear regression $y = ax + b$ with y = mg N/100 g D.M. or mmoles lactate/100 g D.M., x = days after stuffing, a = rate of production and b = calculated initial concentration

[3] 1% of spice mix. 1 added

Table 3 : Effect of different spices on rate and extent of carbohydrate disappearance, lactate production and change in pH[1] (expt. 2)

Spices added	Carbohydrate disappearance			Lactate production			Change in pH		
	b[1]	a[1]	total[2]	a[1]	b[1]	total[2]	b[1]	a[1]	total
none	8.73	- 0.090	3.97	0.52	9.71	4.21	5.77	- 0.006	0.27
Mix. 1	15.05	- 0.429	10.16	1.46	14.44	10.46	5.64	- 0.025	0.94
Black pepper + cloves	11.09	- 0.215	6.84	0.93	10.74	6.82	5.81	- 0.017	0.65
Nutmeg + cinnamon	9.30	- 0.069	3.53	0.48	10.89	3.47	5.73	- 0.006	0.28
Allspice + ginger	11.45	- 0.169	7.17	0.87	14.15	6.21	5.67	- 0.015	0.59
Red pepper + marjoram	9.03	- 0.025	1.43	0.81	9.75	6.08	5.77	- 0.009	0.37
Mustard + mace	11.27	- 0.045	3.30	0.46	11.55	3.36	5.71	- 0.004	0.16

[1] See table 2

[2] mmoles/100 g D.M.