Food Chemistry 30 (1988) 129–135

Off-flavour Reduction in *Vicia faba* Bean Protein Isolate

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(Received 26 August 1987; revised version received and accepted 19 January 1988)

ABSTRACT

Acid gels incorporating protein isolates from Vicia faba *beans have an off-flavour. The odour intensity of this characteristic was quantified on a category scale, and the effect on off-flavour of different methods of protein isolation or addition of flavours to the gels was quantified.*

Analysis of variance (during the protein isolate production) shows that the defatting or isoelectric washing of the bean flour, or the addition of a strawberry aroma concentrate to the gel itself, caused a significant reduction in the off-flavour of the acid bean protein gel. A combination of defatting and isoelectric washing of the bean flour lead to the greatest reduction in off-flavour.

INTRODUCTION

Acceptance by the consumer of new food supplements or raw materials can be achieved only if their functional, as well as their sensory, properties are taken into consideration. The application of an acid-modified protein isolate from *Vicia faba* beans (Schneider *et al.*, 1985; 1986; 1987; Hartmann & Schmandke, 1988), as a substitute for traditional gelling at an acid pH, can give rise to sensory problems as an unpleasant 'fruity' off-flavour develops (Seidel, 1976).

Little information is available on off-flavour formation by faba bean products in an acid pH range.

Seidel (1976) showed that the off-flavour production in faba bean protein

Food Chemistry 0308-8146/88/$03·50

isolates was dependent on the pH-value. At neutral pH a dried pea-like flavour predominated whereas an unpleasant fruity flavour developed in acid conditions. The formation of this fruity flavour depended on protein concentration, time and pH. It was speculated that one or more volatile organic acids produced this specific off-flavour in the faba bean products.

This investigation aimed to find processing methods which produced protein isolates with a reduced intensity of off-flavour.

MATERIALS AND METHODS

Preparation of bean flour and the hydrothermal treatment of beans

Faba beans (*Vicia faba* L. minor, var. 'Erfordia') were either dehulled by a scourer S 1300 (Schneider *et al.*, 1985) or, in addition, were hydrothermally treated for about 5 min at a temperature of 120°C (Schneider *et al.*, 1987). Flour was obtained by milling the treated beans in a centrifugal mill, equipped with a 0·5 mm sieve (Schneider *et al.*, 1986).

Defatting of bean flour

500 g of bean flour were stirred for 30 min at room temperature in 2 litres of isopropanol using a laboratory stirrer. This dispersion was filtered to separate the insoluble residue from the extracted liquid. The extracted bean flour was then dried at room temperature (Schneider *et al.*, 1987).

Isoelectric washing of bean flour

500 g of bean flour, both untreated and defatted, were stirred for 30 min at room temperature in 1·5 litres of tap water, using a laboratory stirrer. The pH of the dispersion was adjusted to 4·2 by adding 2N HCl and maintained during stirring at this level. The dispersion was centrifuged in a laboratory centrifuge, S 70 (VEB Zentrifugenbau Leipzig), for 20 min at 2800 rpm (Schneider *et al.*, 1987). In order to obtain an exhaustive isoelectric washing of the flour the above procedure was repeated four times, taking into account that total mass of dispersion at each washing step had to amount to 2000 g. In both cases (one or four times extracted) the insoluble residue was redispersed after the last washing with a small amount of tap water at pH 4·2 and spray-dried.

Preparation of acid-modified bean protein isolates

300 g of bean flour, both untreated and pretreated (defatting, isoelectric washing, isoelectric exhaustive washing and hydrothermal treatment) were

dispersed in 1·8 litres of tap water using a laboratory stirrer; the pH was adjusted to 7·5 by 2N NaOH.

The protein extraction was completed by further stirring for 30 min. After this extraction the insoluble residue (starch fraction) was separated from the protein extract by centrifugation for 20 min at 2800 rpm. The protein extract was then acidified to pH 2·0 with 2N HCl. The acid was added quickly to reach pH 2·0 within the shortest possible time. The dispersions (pH 2·0) were stirred for about 2 h and then centrifuged under the same conditions as described above. The proteins were isoelectrically precipitated at pH 4·5 from the supernatants by the addition of 2N NaOH (Schneider *et al.*, 1986). Finally, the proteins were redispersed with a small amount of tap water (pH 4·5) and spray-dried.

Preparation of the acid gels

To 20 g of an aqueous solution containing 17·8 g sucrose, 0·75 g citric acid and amaranth colour, 1·74 g acid-modified bean protein isolate was added with stirring. After standing for 3 h at room temperature the mixture was heated in sealed beakers in a water bath for 30 min at 100°C. Immediately after this heat treatment dextrose syrup was added to the fluid mixture. While still hot the mixture was poured into standardized glasses and covered.

When aroma was added, the strawberry aroma concentrate was put into the aqueous solution.

Sensory assessment of odour intensities

It is possible to quantify odour intensities in the same way as other perceived qualities (Torgerson, 1958; Sydow & Petzold, 1981). The acid gels, prepared from acid-modified bean protein isolates, have a specific undesirable fruity off-flavour. The odour intensity of this off-flavour was assessed using a six point category rating scale: not present (0 point), very bland (1), bland (2), moderate (3), strong (4) and very strong (5).

For each test, four panelists were used, who were selected on the basis of their individual differentiation of the odour of practical samples with the described six point category rating scale.

Each of the panelists had to judge three or four different samples per day in intervals of 2 h. Each one of the 18 samples was to be assessed 16 times (4 panelists × 4 repetitions) on different days. The total time to complete all sessions was 18 days.

The statistical evaluation corresponded to the model of Torgerson (Torgerson, 1958; Sydow & Petzold, 1981) by setting the borderline for off-

flavour intensity at 1·5 points (transition of very bland to bland). From the collected data, the frequencies of decisions of <2 and ≥ 2 were determined, using the analog procedure of Gregson (1962), modified by Hoppe & Rödel (1987) (pers. comm.).

The normal deviate z of the relative frequencies of the decisions, served as the final datum of the odour intensities (Torgerson, 1958; Sydow & Petzold, 1981). z indicates the distance of the mean distribution from the selected limen of categories 1·5 ($z = 0$) in units of standard deviation. (Table 1).

TABLE 1
The Normal Deviate z for the Frequencies of the Decision (H) on the Limen of Categories at $n = 16$ Possible Assessments

H		z		H
0	–	∞	+	16
1	–	1·538	+	15
2	–	1·150	+	14
3	–	0·885	+	13
4	–	0·674	+	12
5	–	0·490	+	11
6	–	0·319	+	10
7	–	0·156	+	9
8	–	0	+	8

Negative numerical data (z) show that the mean distribution is placed below 1·5 (limen of categories). Positive numerical data have a mean distribution above 1·5. Therefore, increasing data means increasing off-flavour intensities (Table 1). Data z can be regarded as normally distributed.

RESULTS AND DISCUSSION

Table 2 shows the effects of the experimental variables (effect variables z), strawberry aroma concentrate (I), defatting of bean flour or hydrothermal treatment of beans (II) and aqueous extraction of bean flour at the isoelectric point of the protein (III), on the off-flavour of the bean protein in the gel. In Table 2 the frequencies of the decision ≥ 2 and the resulting calculations of the main actions and interactions are represented additionally. The analysis of variance of the variables (z), gives a residual variance of 0·145 and a standard deviation of 0·380 at 14 degrees of freedom.

Only the mean experimental variables I ($p < 0{\cdot}01$) and II ($p < 0{\cdot}05$) had a significant effect on the off-flavour. There was no interaction between the mean experimental variables.

The analysis of variance after elimination of the experimental variable II 3 (hydrothermal treatment) gives a residual variance of 0·102 and a standard deviation of 0·320 at seven degrees of freedom. In this case the mean experimental variable III (aqueous extraction of bean flour at pH 4·2) also had a significant effect ($p < 0{\cdot}05$) on the off-flavour.

The effect variables and calculations (Table 2) provide the following interpretation:

The addition of strawberry aroma concentrate resulted in a distinct reduction in off-flavour of the protein gels (Table 2, effect calculations of z values of all samples without aroma = 4·559, $\bar{z} = 4{\cdot}559/9 = 0{\cdot}506$; all samples with aroma $= -0{\cdot}559$, $\bar{z} = 0{\cdot}559/9 = -0{\cdot}063$).

The defatting of the bean flour or hydrothermal treatment of the dehulled beans had different effects on the off-flavour of the gels. In comparison with the untreated bean flour, defatting led to a reduction in the off-flavour. On the other hand, hydrothermal treatment resulted in an increase in the gel off-flavour (Table 2, effect calculation of z-values of all defatted samples $= -0{\cdot}729$, $\bar{z} = -0{\cdot}729/6 = -0{\cdot}122$; all untreated samples = 0·809, $\bar{z} = 0{\cdot}809/6 = 0{\cdot}135$; all hydrothermal treated samples = 3·190, $\bar{z} = 3{\cdot}190/6 = 0{\cdot}652$).

Aqueous extraction of the bean flour at pH 4·2 reduced the off-flavour; this reduction was more apparent after exhaustive extraction. However, when aqueous extraction was combined with hydrothermal treatment, no reduction in off-odour was detected. (Effect calculations of the analysis of variance without consideration of the experimental variable II 3; Table 2, effect sums of z-values of all untreated samples = 1·292, $\bar{z} = 1{\cdot}292/4 = 0{\cdot}323$; all one-time-extracted samples = 0·347, $\bar{z} = 0{\cdot}347/4 = 0{\cdot}087$; all exhaustive extracted samples $= -1{\cdot}559$, $\bar{z} = -1{\cdot}559/4 = -0{\cdot}390$).

Experiments to reduce the off-flavour components not only in bean flour but also in bean protein isolates (by means of ultrafiltration or enzymatic treatment) were not successful.

CONCLUSIONS

These results show that it is possible to assess off-flavour without having knowledge of the identity of the component causing the off-flavour. The quantification of the off-flavour in different faba bean flours and the

TABLE 2

Results of the Off-Flavour Judgement of Faba Bean Protein Gels prepared from Different Acid Modified Faba Bean Protein Isolates (II and III) respectively by Additional Use of a Strawberry Aroma Concentrate (I)

Gel No.	Experimental variables			z	Effect variables and sums					
	I	II	III		II × III	I × III	III	I × II	II	I
1			1	0·490		1·796	2·761			
				11/16[a]			1·292[b]			
								1·483	0·809	
2		1	2	0·674		1·878	1·232			
				12/16			0·347[b]		0·809[b]	
3			3	0·319		0·885	0·000			
				10/16			−1·559[b]			
4			1	0·156						
	1			9/16						
5		2	2	0·319				0·156	−0·729[b]	4·559
				10/16					−0·729[b]	1·639[a]
6			3	−0·319						
				6/16						
7			1	1·159						
				14/16						
8		3	2	0·885				2·920	3·913	
				13/16						
9			3	0·885						
				13/16						
10			1	0·490	0·980	0·965				
				11/16						
11		1	2	−0·490	0·184	−0·646		−0·674		
				5/16						
12			3	−0·674	−0·355	−0·885				
				4/16						
13			1	0·156	0·312					−0·566[a]
				9/16						−1·403[a]
14	2	2	2	−0·156	0·163			−0·885		
				7/16						
15			3	−0·885	−1·204					
				3/16						
16			1	0·319	1·469					
				10/16						
17		3	2	0·000	0·885			0·993		
				8/16						
18			3	0·674	1·559					
				12/16						

[a] Frequency of the judgement decision for intensities ≥2.

[b] Effect calculations of the analysis of variance without consideration of the experimental variable II_3.

Experimental variable I addition of strawberry aroma concentrate.
- I_1 without aroma.
- I_2 with aroma.

Experimental variable II preparation of faba bean flour.
- II_1 untreated flour.
- II_2 defatted flour.
- II_3 flour of hydrothermal treated faba beans.

Experimental variable III aqueous extraction of faba bean flour at pH 4·2.
- III_1 unextracted flour.
- III_2 one true extracted flour.
- III_3 exhaustively extracted flour.

corresponding faba bean protein isolates was determined using a category scale to assess the intensity of odour from gels prepared with faba bean protein isolates. In this way it was possible to find out the effectiveness of various processing methods on the reduction of the off-flavour.

Neither the addition of strawberry concentrate nor defatting or hydrothermal treatments, alone or in combination, led to a total removal of the undesirable off-flavour. Off-flavour in the gel was only reduced below the 1·5 level (very bland to bland) when the flour had been defatted and exhaustively extracted with water at pH 4·2 ($z = -0{\cdot}319$). When strawberry concentrate was added to this gel the off-odour was further reduced ($z = 0{\cdot}885$). The off-odour of the latter approached that of a standard fruit gel ($z = -1{\cdot}150$), prepared with agar-agar as a gelling agent.

The off-flavour components of faba beans and their processed products originate mainly in the lipid fraction (Hinchcliffe *et al.*, 1977). The special off-flavour component in acid pH is largely extractable at pH 4·2 (isoelectric point of the faba bean protein). Therefore, Seidel (1976) supposed organic acids to be the off-flavour components.

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