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EFFECT OF PRE-FREEZING AND CULINARY TREATMENT ON THE CONTENT OF AMINO ACIDS OF GREEN PEA

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Abstract. Green pea is regarded as an important constituent of a human diet, especially for vegetarians. The aim of the present work was to evaluate the amino acids content in green pea and the quality of pea protein. The study covered raw seeds; fresh seeds cooked to consumption consistency; and two kinds of frozen products prepared for consumption: frozen seeds obtained using the traditional method and frozen seeds of the ready-to-eat type. Compared with the raw material, cooked fresh pea contained more isoleucine (15%), valine (14%) and arginine (24%) but less tyrosine (17%); cooked pea from the traditional frozen product contained less sulphur-containing amino acids (12%) and alanine (13%); while pea from the frozen product of the ready-to-eat type contained a similar or higher amount (from 12% to 38%) of amino acids, except for sulphur-containing amino acids (less 12%). The protein of green pea was of very good quality, both in raw seeds and in those prepared for consumption. In comparison with the FAO/WHO/1991 standard, the CS indexes exceeded 100. It was only for sulphuric amino acids that the CS for the ready-to-eat product was 98. The methods of culinary and technological processing applied affected the quality of protein in green pea seeds to a negligible degree.

Key words: green pea, blanching, cooking, freezing, microwave thawing and heating

INTRODUCTION

Pulses, once known as the poor man's meat, are nowadays regarded as important constituents of a healthy diet. They are also a staple food of vegetarians [Leterme 2002]. Regular consumption of pulses can prevent heart disease, rectal and breast carcinoma as well as other illnesses [Anderson and Major 2002, Mathers 2002]. According to nutritionists, in spite of a slight increase in the consumption of pulses in Europe in recent years, the level still remains too low [Schneider 2002].

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The seeds of pulse crops tend to be used after they have reached full physiological maturity. However, some species are consumed when not quite ripe, at what is called milk-stage or wax-stage. At this stage – unlike physiologically mature seeds – the keeping quality of the seeds is poor and they have to be processed. Among this group are: pea; broad bean; flageolet bean; and soybean, but only vegetable type. In European countries, pea is the most important of these species and its processing generally takes the form of canning or freezing.

Owing to the growing interest of consumers in products of the "do-it-for-me" and "ready-to-eat" type, their production and supply have increased rapidly [Sloan 2005]. This type of product requires the producer to carry out certain culinary procedures thereby limiting the role of the consumer in the preparation of the dish to heating, an easily accomplished task using a microwave oven [Datta et al. 2005].

The aims of the present work were to evaluate: the amino acids content in products obtained from green pea seeds at the stage of milk maturity; and the quality of pea protein. The study covered raw seeds; seeds cooked to consumption consistency; and two kinds of frozen products prepared for consumption: frozen pea obtained using the traditional method (seeds blanched before freezing) and frozen seeds of the ready-to-eat type (seeds cooked before freezing).

MATERIAL AND METHODS

Material

The studied material consisted of fresh pea seeds (*Pisum sativum*) as the raw material, fresh seeds cooked to consumption consistency, and frozen pea prepared for consumption after 12 months of frozen storage. The cultivar Consul (Van Waveren Saaten) was used in the study. The harvest was carried out when the dry matter was 23.37 g/100 g fresh matter. The seeds were shelled and sorted by size, the seeds taken for processing being 9-11 mm in diameter.

Methods

Production of frozen products

Two variants were used in preparing the raw material for freezing. Using traditional technology, variant I, the raw material was blanched, and after freezing and storage the frozen green peas needed traditional cooking. In variant II, the raw material was cooked before freezing to a condition approximating to consumption consistency, hence the obtained ready-to-eat product merely required defrosting and heating in a microwave oven.

In variant I, pea seeds were blanched in a stainless steel vessel in water, the proportion of the blanched material to water being 1:5. The blanching temperature was 95--98°C and the time was 2 min 30 sec. These conditions permitted a decrease in the activity of catalase and peroxidase to a level below 5% of the initial value (unpublished data). After blanching the material was immediately cooled in cold water, slightly shaken and left for 30 min on sieves to drain the water remaining on the surface.

In variant II, pea seeds were cooked to a condition approximating to consumption consistency in 2% (w/w) brine. The cooking was carried out in a stainless steel vessel and the proportion of the raw material to brine was 1:1 (w/w). The pea seeds were than placed in boiling water. The time of cooking measured from the moment when the medium came to the boil, to the moment the desired consistency was obtained was 8 min. After cooking the material was drained, placed in sieves and cooled in a stream of cold air.

The material from the blanched and cooked samples was placed on trays and frozen at –40°C in a Feutron blast freezer (Greiz, Germany), type 3626-51, to –20°C, which was obtained inside the frozen product after 90 min. After the desired temperature was obtained, 500 g portions of the seeds were packed in polyethylene bags suitable for the storage of frozen products. The bags were placed in chamber freezers at –20°C.

The preparation of frozen green pea for evaluation

Frozen product prepared from blanched material was cooked in a brine containing 2% (w/w) of table salt (NaCl), the proportion in weight of brine to pea seeds being 1:1 (w/w). As in the case of material cooked before freezing, the material was put in boiling brine containing 2% (w/w) of table salt. The time of cooking was 6 min measured from the moment when the brine came to the boil. After cooking the water was immediately drained and the product was cooled to 20°C for analyses.

Frozen pea products cooked before freezing were heated in a heat-resisting vessel covered with a lid in a Panasonic microwave type NN-F621. The time of defrosting and heating to 75°C [Codex Alimentarius 1993] was 7 min 45 s.

Analytical procedures

The content of dry matter and total nitrogen (total N) were determined according to procedures described by the Association of Official Analytical Chemists [AOAC 1990]. The content of amino acids was determined using an AAA-400 amino acid analyser (INGOS, the Czech Republic). Content of the tryptophan was not measured. As Iqbal et al. [2006] showed the level of the tryptophan in green pea was 0.8% of total amino acids, so its quantity in small degree effect on the content of total amino acids. The analytical procedure applied was in accordance with the recommendations of the producer. The freeze-dried material was hydrolysed in 6 M HCl for 24 h at 110°C. After cooling, filtering and washing, the hydrolyte was evaporated in a vacuum evaporator, the dry residue being dissolved in a citrate buffer of pH 2.2. The prepared sample was analysed using the ninhydrine method. Citrate buffers of pH 2.6; 3.0; 4.25; and 7.9 were applied. The ninhydrine solution was buffered in acetate buffer at pH 5.5. A column 370 mm in length was filled with Ostion ANB INGOS ionex (the Czech Republic). The temperature of the column was 55-74°C; that of the reactor 120°C. Preparation by acid hydrolysis includes glutamine and asparagine for glutamic acid and aspartic acid respectively. The determination of the sulphur-containing amino acids, methionine and cystine, was carried out by means of oxygenating hydrolysis, using a mixture of formic acid and hydrogen peroxide (9:1) at 110°C for 24 h. After cooling, the sample was processed as with acid hydrolysis (as described above). Citrate buffers of pH 2.6 and 3.0 were used; the temperature of the column was 60°C and that of the reactor 120°C. The calculations were carried out according to the external standard.

Expression of results

The level of amino acids was given in 100 g of edible parts of the products in order to compare the amino acid content in pea seeds according to the culinary and technological processing applied.

The composition of amino acids was also expressed as grams per 16 g of N to estimate the quality of the protein in pea seeds by comparing it with the FAO/WHO pattern [FAO/WHO 1991, Protein... 2002]. On the basis of the amino acid composition, the chemical score (CS) index which is used in defining limiting amino acid was calculated using the Mitchell and Block method [Osborne and Voogt 1978], and the integrated essential amino acid (EAA) index using the Oser method [Oser 1951].

Statistical analysis

Statistical analysis allowing a comparison of the content of amino acids in the fresh raw material, cooked raw material and frozen goods after preparation for consumption was carried out using single-factor analysis of variance (ANOVA) on the basis of the Snedecor *F*-distribution and Student's t tests, and the least significant difference (LSD) was calculated at the probability level $\alpha = 0.01$ and $\alpha = 0.05$ [Snedecor and Cochman 1980]. The Statistica 6.1 (StatSoft Inc., Tulsa, OK, USA) program was applied.

RESULTS AND DISCUSSION

The composition of total amino acid in raw green pea seeds was as follows: glutamic and aspartic acids over 10%; leucine, lysine and arginine 8-9%; phenylalanine, threonine, valine, serine, proline, glycine and alanine 5-6%; and isoleucine, tyrosine and histidine 3-4%. The lowest values were recorded for methionine and cystine: respectively 1.4% and 1.7% in total amino acids and 3.2% and 3.4% in total essential amino acids (Table 1). Similar results, as in present work, were obtained by Lee et al. [1982] and Iqbal et al. [2006]; it should be noted that Lee et al. [1982] analysed pea seeds of a similar stage of maturity because dry matter was 20.92%, while Iqbal et al. [2006] studied physiologically mature seeds. In presented work the amino acids content in pea cooked directly after harvest differed only slightly. Compared with the raw material, in 100 g of the cooked product the content of isoleucine, valine and arginine was higher ($\alpha = 0.01$), that of tyrosine lower ($\alpha = 0.01$) and that of threonine and serine as well as total aromatic amino acids lower at $\alpha = 0.05$.

In our study the products prepared for consumption from frozen pea after storage differed from the raw material to a slightly greater degree with respect to amino acid content. Compared with the raw material, in frozen pea obtained using the traditional method, i.e. blanched before freezing, the content of total sulphuric amino acids and alanine was lower at $\alpha = 0.01$ and of both sulphuric amino acids, tyrosine, total aromatic amino acids, threonine and glycine at $\alpha = 0.05$. However, total amino acid content did not change significantly. Compared with the raw material, the ready-to-eat product, i.e. cooked before freezing, contained less ($\alpha = 0.05$) sulphuric amino acids but more ($\alpha = 0.01$) isoleucine, phenylalanine, valine, arginine and total non-essential amino acids. It also contained more ($\alpha = 0.05$) leucine, lysine, glutamic acid and also total essential amino acids and total amino acids.

Table 1. Amino acid composition of raw and processed pea, mg/100 g of product (means ±standard deviation)

Amino acid	Seeds		Goods prepared for consumption from frozen seeds	
	raw	cooked	blanched before freezing	cooked before freezing
Isoleucine	271 ±12^{Aa}	312 ±13^{BCbc}	291 ±18^{ABab}	330 ±17^{Cc}
Leucine	519 ±24^{ABa}	515 ±25^{ABa}	494 ±18^{Aa}	557 ±17^{Bb}
Lysine	526 ±22^{ABa}	524 ±28^{Aa}	500 ±20^{Aa}	574 ±20^{Bb}
Cystine	110 ±4^{ABb}	113 ±6^{Bb}	99 ±5^{Aa}	98 ±7^{Aa}
Methionine	93 ±4^{Ac}	88 ±8^{Abc}	79 ±5^{Aa}	80 ±5^{Aab}
Total sulphur amino acids	203^{Bb}	201^{Ab}	178^{Aa}	178^{Aa}
Tyrosine	228 ±13^{Cc}	190 ±11^{Aa}	201 ±12A^{Cab}	217 ±12^{BCbc}
Phenylalanine	331 ±18^{Aa}	329 ±15^{Aa}	316 ±16^{Aa}	371 ±20^{Bb}
Total aromatic amino acids	559A^{Bb}	519^{Aa}	517^{Aa}	588^{Bb}
Threonine	313 ±17^{Ac}	276 ±17^{Aab}	268 ±14^{Aa}	298 ±21^{Abc}
Valine	311 ±18^{Aa}	355 ±19^{Bbc}	326 ±22^{ABab}	367 ±21^{Bc}
Histidine	183 ±6A^{Bab}	177 ±10^{ABa}	169 ±9^{Aa}	196 ±10^{Bb}
Total essential amino acids	2 885^{Aa}	2 879^{Aa}	2 743^{Aa}	3 088^{Ab}
Arginine	572 ±32^{Aa}	712 ±33^{Bb}	579 ±80^{Aa}	787 ±50^{Bb}
Aspartic acid	740 ±26^{Aa}	715 ±36^{Aa}	692 ±28^{Aa}	732 ±33^{Aa}
Glutamic acid	1 049 ±64^{Aa}	1 098 ±62^{Aab}	1 039 ±62^{Aa}	1 171 ±65^{Ab}
Serine	346 ±15^{Ab}	301 ±17^{Aa}	319 ±20^{Aab}	339 ±24^{Ab}
Proline	298 ±13^{Aa}	291 ±9^{Aa}	279 ±12^{Aa}	300 ±11^{Aa}
Glycine	299 ±15^{Ac}	290 ±14^{Aab}	274 ±15^{Aa}	311 ±13^{Abc}
Alanine	382 ±24^{ABbc}	360 ±22^{Bab}	331 ±17^{Aa}	394 ±16^{Bc}
Total non-essential amino acids	3 686^{Aa}	3 767^{Aab}	3 513^{Aa}	4 034^{Ab}
Total amino acids	6 571^{Aa}	6 646^{Aab}	6 256^{Aa}	7 122^{Ab}
Dry matter g/100 g of product	23.37	24.23	23.36	26.34

The data reported are means of three independent replicate analyses for two samples.

Means in the same row with different folowing superscript capital letters are significantly different at $\alpha = 0.01$ and with small letters at $\alpha = 0.05$.

Comparing the two types of frozen product prepared for consumption, that obtained from pea cooked before freezing contained higher amounts of most amino acids at $\alpha = 0.01$ or $\alpha = 0.05$ than that obtained from traditionally frozen pea. There were no significant differences in the levels of sulphuric amino acids, tyrosine, aspartic acid, serine or proline. The higher amino acid content in the ready-to-eat product could be due to its dry matter content, being 13% higher than in the traditionally frozen product (Table 1). Other works have also shown that a frozen product prepared for consumption in a microwave oven contained more dry matter than one cooked in water [Gębczyński 2006, Gębczyński and Lisiewska 2006, Lisiewska et al. 2007]. Thus, in expressing the content of amino acids per 100 g of product, the level could have been affected by the "dilution" or "concentration" of constituents, which in turn would depend on the leaching of soluble constituents and the absorption of water by the starch contained in the seeds [Kmiecik et al. 2004, Ruiter et al. 1984].

Lee et al. [1982] showed that the retention of amino acids in green pea seeds depended on the temperature and time of thermal treatment; at low levels of processing, the amino acid content was stable, while with more intensive processing the content of alanine, threonine and serine was significantly reduced. According to Candela et al. [1997] with identical processing parameters change, loss or increase in amino acid composition depended on the species of pulse. Similarly, Lisiewska and Kmiecik [1990] and Lisiewska et al. [1999] showed that within a single species changes in amino acid content depended on the cultivar. Lanfermeijer et al. [1989] and Ruiter et al. [1984] stress that the loss of amino acids depends, among other things, on possible damage to the seed coat, the release of amino acids from the cotyledons, and also from leakage to the bathing medium resulting from solubility in an aquatic environment. Moreover, as the cited authors claim, the younger the seeds, the greater the loss. The pea seeds considered in the present work were those at the most appropriate stage of maturity for the processing industry.

Expressing the amino acid content per 16 g N, the changes brought about by cooking fresh pea seeds and the preparation of seeds for consumption after frozen storage were much lower (Table 2). The cooking of raw seeds increased the content of isoleucine and arginine at $\alpha = 0.01$ and of valine at $\alpha = 0.05$; but caused a decrease ($\alpha = 0.01$) in tyrosine, ($\alpha = 0.05$) threonine and serine. Compared with the raw material, the product obtained by cooking frozen pea produced using the traditional method (blanching before freezing) contained more ($\alpha = 0.01$) isoleucine and ($\alpha = 0.05$) valine and less ($\alpha = 0.05$) methionine and threonine. As with the above mentioned products, pea cooked before freezing, then defrosted and heated in a microwave oven contained more isoleucine and, as in the case of pea cooked from the raw material, more arginine but lower ($\alpha = 0.01$) amounts of both sulphuric amino acids and at $\alpha = 0.05$ less tyrosine, threonine and serine.

In an investigation by Khalil and Mansour [1995], culinary processing did not change the level of total essential amino acids in the protein of broad bean seeds irrespective of the parameters applied, while an increase was recorded in the content of leucine, threonine and histidine. Different results were obtained by Chau et al. [1997] in cooking three species of pulse. These authors found a decrease in most essential amino acids; however, the behaviour of different species was not uniform. Porres et al. [2002] found an increase in the content of leucine, lysine and phenylalanine with tyrosine during the thermal processing of lentils in water.

As in the present study, Wu et al. [1996] reported that, in spite of various changes in the content of amino acids depending on the species and the parameters of thermal processing, lysine, valine, serine and proline were fully stable ($\alpha = 0.01$). However, Clemente et al. [1998] report that, unlike in the present study of green pea, the cooking of chickpea reduced the content of arginine, cystine, leucine and lysine. Neither was

Table 2. Amino acid composition of raw and processed pea, in grams per 16 g of N (means ±standard deviation)

Amino acid	Seeds		Goods prepared for consumption from frozen seeds	
	raw	cooked	blanched b efore freezing	cooked before freezing
Isoleucine	3.99 ±0.17^{Aa}	4.61 ±0.19^{Bb}	4.54 ±0.28^{Bb}	4.51 ±0.24^{Bb}
Leucine	7.66 ±0.36^{Aa}	7.62 ±0.36^{Aa}	7.70 ±0.27^{Aa}	7.62 ±0.23^{Aa}
Lysine	7.76 ±0.32^{Aa}	7.75 ±0.41^{Aa}	7.80 ±0.31^{Aa}	7.85 ±0.27^{Aa}
Cystine	1.62 ±0.06^{Bb}	1.68 ±0.08^{Bb}	1.55 ±0.08^{Bb}	1.35 ±0.09^{Aa}
Methionine	1.37 ±0.05^{Cc}	1.30 ±0.12^{Cbc}	1.23 ±0.07^{BCb}	1.10 ±0.07^{ABa}
Total sulphur amino acids	2.99^{Bb}	2.98^{Bb}	2.78^{ABb}	2.45^{Aa}
Tyrosine	3.36 ±0.20^{Bb}	2.81 ±0.17^{Aa}	3.14 ±0.18^{ABbc}	2.97 ±0.17^{ABac}
Phenylalanine	4.88 ±0.26^{Aa}	4.86 ±0.23^{Aa}	4.93 ±0.24^{Aa}	5.07 ±0.27^{Aa}
Total aromatic amino acids	8.24^{Aa}	7.67^{Aa}	8.07^{Aa}	8.04^{Aa}
Threonine	4.62 ±0.25^{Ab}	4.08 ±0.25^{Aa}	4.17 ±0.21^{Aa}	4.08 ±0.29^{Aa}
Valine	4.59 ±0.26^{Aa}	5.25 ±0.28^{Ab}	5.08 ±0.35^{Ab}	5.01 ±0.28^{Aab}
Histidine	2.69 ±0.09^{Aa}	2.62 ±0.15^{Aa}	2.64 ±0.13^{Aa}	2.68 ±0.13^{Aa}
Total essential amino acids	42.54^{Aa}	42.58^{Aa}	42.78^{Aa}	42.24^{Aa}
Arginine	8.44 ±0.47^{Aa}	10.54 ±0.49^{Bc}	9.03 ±1.24^{Ab}	10.77 ±0.68^{Bc}
Aspartic acid	10.92 ±0.39^{Aa}	10.57 ±0.53^{Aa}	10.80 ±0.43^{Aa}	10.02 ±0.45^{Aa}
Glutamic acid	15.47 ±0.95^{Aa}	16.24 ±0.92^{Aa}	16.21 ±0.97^{Aa}	16.03 ±0.89^{Aa}
Serine	5.10 ±0.21^{Ac}	4.45 ±0.25^{Aa}	4.97 ±0.32^{Abc}	4.64 ±0.33^{AAab}
Proline	4.40 ±0.19^{Aa}	4.31 ±0.13^{Aa}	4.36 ±0.19^{Aa}	4.10 ±0.15^{Aa}
Glycine	4.41 ±0.22^{Aa}	4.30 ±0.21^{Aa}	4.27 ±0.24^{Aa}	4.26 ±0.18^{Aa}
Alanine	5.64 ±0.35^{Aa}	5.32 ±0.32^{Aa}	5.17 ±0.27^{Aa}	5.39 ±0.21^{Aa}
Total non-essential amino acids	54.38^{Aa}	55.73^{Aa}	54.81^{Aa}	55.21^{Aa}
Total amino acids	96.92^{Aa}	98.31^{Aa}	97.59^{Aa}	97.45^{Aa}
Total N, g/100 g fresh matter	1.09	1.08	1.03	1.17

The data reported are means of three independent replicate analyses for two samples.

Means in the same row with different folowing superscript capital letters are significantly different at α = 0.01 and with small letters at α = 0.05.

there confirmation of the results reported by El-Adawy [2002], who observed that cooking in water and in a microwave oven brought about a small increase in the content of total essential amino acids.

In our paper, a comparison of the value of both products obtained from frozen pea showed that the ready-to-eat type contained more (α = 0.01) arginine and less sulphuric amino acids, cystine (α = 0.01) and methionine (α = 0.05). In pea seeds non-essential amino acids predominated over essential amino acids, as shown by the ratio of essential amino acids to non-essential amino acids. It was similar for the studied samples, varying in the range of 1.28-1.31 (Table 2).

The protein of green pea, investigated in the present work, was of very good quality, both in raw seeds and in those prepared for consumption. In comparison with the FAO/WHO [1991] standard, the CS index exceeded 100. It was only for sulphuric amino acids that the CS for the ready-to-eat product was 98 (Table 3). Since the standards for ideal protein have changed over time [Pysz and Pisulewski 2004], it is hard to compare the CS values obtained with the results of other authors. The computed values of the EAA index were very similar to all the studied samples 129-132, showing that the methods of culinary and technological processing applied affected the quality of protein in green pea seeds to a negligible degree. However, as Gębczyński [2006] reports, green pea prepared for consumption from the frozen product obtained using the modified technology (cooking before freezing) contained higher amounts of polyphenols and beta-carotene and had higher antioxidative activity than cooked pea obtained from the traditional frozen product.

Table 3. Amino acids indexes of raw and processed pea according to FAO/WHO (1991)

Index	Amino acid	Seeds		Goods prepared for consumption from frozen seeds	
		raw	cooked	blanched before freezing	cooked before freezing
CS	Isoleucine	143	165	162	161
	Leucine	116	115	117	115
	Lysine	134	134	134	135
	Cystine with methionine	120	119	111	98
	Tyrosine with phenylalanine	131	122	128	128
	Threonine	136	120	123	120
	Valine	131	150	145	143
	Histidine	142	138	139	141
EAA		131	132	132	129

CS – Chemical Score index.
EAA – Essential Amino Acid index.

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WPŁYW OBRÓBKI WSTĘPNEJ PRZED MROŻENIEM ORAZ PRZYGOTOWANIA DO SPOŻYCIA NA ZAWARTOŚĆ AMINOKWASÓW W ZIELONYM GROCHU

Streszczenie. Celem pracy była ocena zawartości aminokwasów w produktach z nasion grochu zielonego o dojrzałości mlecznej oraz ocena jakości białka grochu. W badaniach uwzględniono nasiona surowe, nasiona ugotowane do konsystencji konsumpcyjnej oraz dwa rodzaje mrożonek po przygotowaniu do konsumpcji, w tym mrożonkę otrzymaną sposobem tradycyjnym (nasiona blanszowane przed mrożeniem) i mrożonkę typu żywności wygodnej (nasiona gotowane przed mrożeniem). W porównaniu z nasionami surowymi groch ugotowany miał więcej izoleucyny, waliny i argininy oraz mniej tyrozyny; groch ugotowany z mrożonki otrzymanej sposobem tradycyjnym charakteryzował się mniejszą ilością aminokwasów siarkowych i alaniny; groch przygotowany do spożycia z mrożonki typu żywność wygodna wyróżniał się podobną lub większą ilością aminokwasów, z wyjątkiem aminokwasów siarkowych. Wyrażenie zawartości aminokwasów w 16 g N wykazało znacznie mniejsze zmiany spowodowane gotowaniem nasion świeżych i zabiegami związanymi z przygotowaniem grochu do konsumpcji z mrożonek. Aminokwasem ograniczającym była cystyna z metioniną.

Słowa kluczowe: groch zielony, blanszowanie, gotowanie, mrożenie, rozmrażanie i ogrzewanie mikrofalowe

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